

CAMBRIDGE UNIVERSITY PRESS  
LONDON: FETTER LANE, E.C. 4



H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1  
WHELDON & WESLEY, LTD., 2-4, ARTHUR ST., NEW OXFORD ST., LONDON, W.C. 2

CHICAGO: THE UNIVERSITY OF CHICAGO PRESS  
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# The British Mycological Society

*(Recognosce notum, ignotum inspice)*

## TRANSACTIONS

### Volume XI

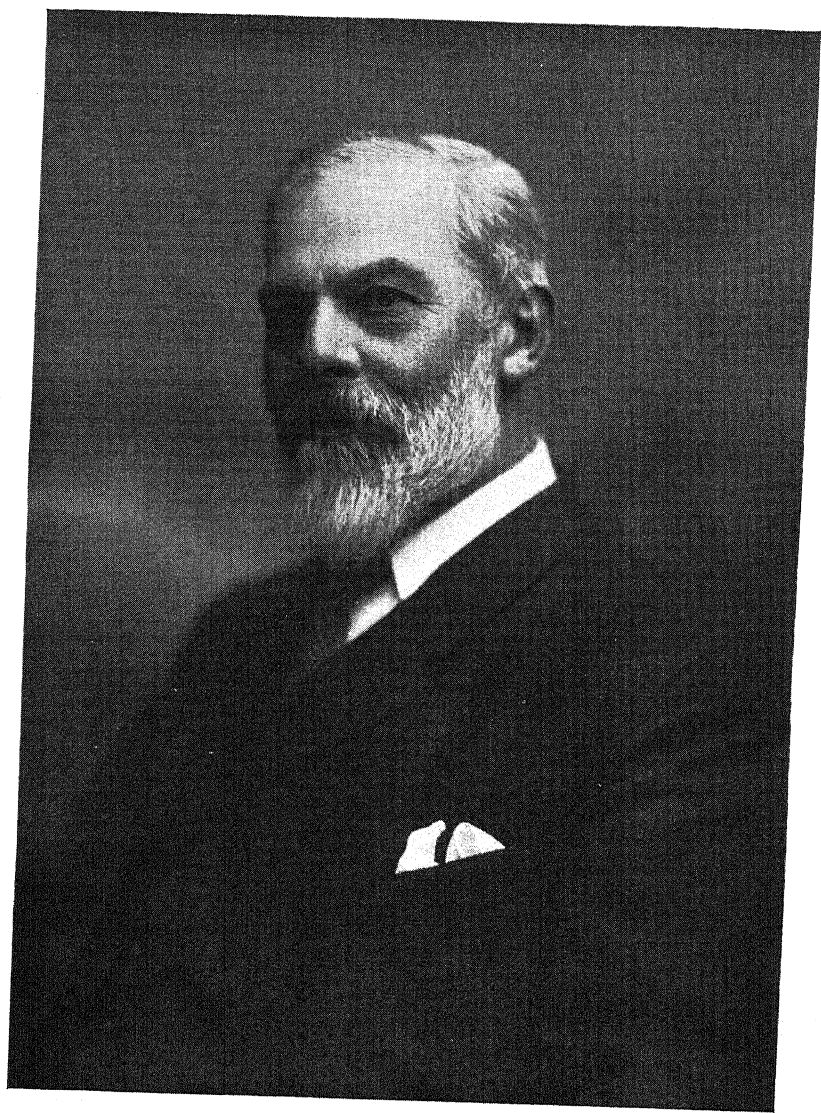
Edited by

CARLETON REA and J. RAMSBOTTOM

CAMBRIDGE  
AT THE UNIVERSITY PRESS  
1926



PRINTED IN GREAT BRITAIN



WILLIAM NORWOOD CHEESMAN



## WILLIAM NORWOOD CHEESMAN.

1847-1925.

By J. Ramsbottom.

By the death of Mr W. N. Cheesman, J.P., F.L.S., our Society, for the first time in its history, had to mourn the loss of the President during his term of office. He had been ill for some months but the end, when it came, was unexpected.

William Norwood Cheesman was born at Winterton, Lincs. on Feb. 1st, 1847; as a youth he spent some time at Hull and then went to London to learn the drapery business. In his twenty-fifth year he joined his uncle at Selby in the extensive drapery establishment which later became Cheesman (Selby) Ltd. Latterly he had retired from active participation in its affairs though he retained his seat on the directorate. It was while in London that Mr Cheesman began to take an interest in nature study. The controversies which had not then died down after the publication of Darwin's *Origin of Species* no doubt gave many young men their first taste for natural history or whetted their appetites. That it may have been so in Cheesman's case also seems likely as he was proud of having known Charles Darwin whose home at Mitcham he had visited occasionally.

On going to Selby (1870) young Cheesman soon got a local reputation as a general naturalist, but particularly as a botanist. In the first volume of the new series\* of the *Naturalist* (1875-6), the Journal of the West Riding Consolidated Naturalists' Societies which was formed in 1861, he is shown as Secretary of the Selby Naturalists' Society. With the joining up of local societies as the Yorkshire Naturalists' Union (1877) a comprehensive study of the natural history of the county was initiated. After the time of James Bolton's *History of Fungusses growing about Halifax* (1788-91) the study of fungi in Yorkshire had been spasmodic. Now a number of members, among whom were Dr H. F. Parsons, Rev. W. Fowler, Messrs W. West and W. N. Cheesman interested themselves in fungi as one of the "neglected orders" which were marked out for special study. A fungus foray took place in 1881 with Leeds as a centre. The next foray was held at Selby in 1884, the arrangements being made by Mr Cheesman: the account of this foray (*Naturalist*, x, p. 140, 1885) suggests that hospitality on his part was not lacking. A third foray was held in 1888 and since 1891 there has been one annually. The study of fungi in the field was increasing

\* The first series was 1864-67.

in popularity in Yorkshire at a time when the Woolhope forays were declining after Dr Bull's death in 1886. A mycological committee was formed in 1892. "The justly celebrated Hereford Foray...the universally acknowledged meeting place for exchange of opinion and courteous criticism between British and foreign mycologists has unfortunately run its course, and it is the hope and ambition of the Yorkshire Union that the annual autumn gathering may—by avoiding the weak points of its predecessor, which were mainly confined to an excess of hospitality—prove at least equally attractive and instructive to mycologists"\*.

At the Huddersfield foray in 1895 the idea of a National Mycological Union was discussed and at Selby the following year the British Mycological Society was formed, the arrangements being talked over in Cheesman's house, "over a cup of tea" to use his own words. Mr George Massee was elected first President and held the post for two years; then for some reason he withdrew from the Society with several of his Yorkshire friends, among whom was his great admirer Mr Cheesman. What Massee failed to realise was that the day of supreme authority had passed, even in systematic mycology, but he was willingly accorded that position in Yorkshire, continuing as chairman of the Mycological Committee until his death. When it was clear that the British Mycological Society was accomplishing the work it had set itself, matters straightened out to a great extent and at the time I joined the Society in 1910 there was no more enthusiastic member than Mr Cheesman. I met him for the first time at the Taunton foray in 1911. He acted there as he always did with beginners, taking the greatest delight in pointing out the characters of the fungi exhibited on the plates: one had only to show interest in order to receive every assistance it was in his power to give. Many mycologists on looking back at their first foray will doubtless recall that it was our late President who made it his business to give them what help he could. He did not enjoy the reputation of some of his Yorkshire colleagues as a systematist—many of them acquired European reputations—but his work did not lie in that direction. He was essentially a populariser of the subject and as a careful and energetic collector he was one of the best-known amateurs in the country. In later years he was particularly interested in Mycetozoa.

As a recognition of his efforts in furthering the study of mycology he was elected President of the Yorkshire Naturalists' Union in 1916 and took as the subject for his address *Economic Mycology: the beneficial and injurious influences of fungi*. The

\* C. Crossland, *Naturalist* (1894), p. 69.

title was fully indicative of the scope of the address which was an able exposition of the importance of the study of fungi.

He was a regular attender at the British Association meetings both at home and overseas. In this country he represented our Society as delegate on several occasions: his visits further afield to Canada, South Africa, Australia and New Zealand resulted in excellent collections of fungi and Mycetozoa.

In 1918 he was elected to the newly formed Council\* of our Society, served as Vice-president in 1923, and was elected President for the year 1925.

He felt that his health might prevent him from being an effective President but arrangements were made that enabled him to consent to serve. He fully appreciated the honour judging from the letters he wrote in answer to accounts he received of arrangements to be made and those carried out. It was a great trouble to him that he was not well enough to prepare a Presidential address, but it was a medical veto rather than the advice of his friends that at length persuaded him that it would be unwise to attempt one. Just before the Dublin foray he wrote to me as follows:

"I have just received the notice of our annual meeting at Dublin and I regret very much that I am not able to attend; I am still having the doctor's attention; at times I think that I am getting better at other times I think otherwise.

"Certainly I am better than I was a year ago, but am only able to walk about gently with my stick; my time is spent all day in my garden and greenhouse, when I am able to ponder over the pleasant past and the joyous reflections of happy days spent in the fields and at our Forays. I am thus able, in contentment, to pass my time smoking my pipe (four ounces a week), working crossword puzzles, solving chess problems and reading light, frivolous literature....

"I had two or three plans for my address to the members but my doctor strongly discouraged me from undertaking any serious mental work.

"Please convey to the members my hearty greetings and my grateful thanks for the great honour they did me by electing me as their President. I am now sorry that my illness has made me such a useless President.

"I hope that you will have a good time at Dublin and find plenty of interesting 'stuff.'

"My best wishes are for the continued prosperity of the Society and my hope that the membership will soon number a thousand for with increased membership the hands of the officers will thereby be strengthened in the important work they are carrying on.

\* Again at Selby.

"The B.M.S. Forays have been bright periods in my life and many other members will have the same feelings.

"Again, with best wishes to all,

"I remain, Yours sincerely,

"W. N. Cheesman.

"P.S. I enclose cheque for one hundred guineas which I wish our Treasurer to invest and the proceeds applied to the printing fund or to such other purposes as the Council may decide."

This unexpected, though characteristic generosity, was greatly appreciated. After considerable discussion the Council decided that the best use of the gift would be to enable one or more students to attend the autumn forays which our President so valued. It was decided to ask him whether this would meet with his approval—but he died on November 7th, the day following the meeting.

In addition to his natural history activities Mr Cheesman studied Archaeology and was for many years a prominent member of the Yorkshire Archaeological Society. He was keenly interested in Selby Abbey and acted as churchwarden for some years. As a prominent Freemason he combined his archaeology and his craft and wrote several papers, the most important of which was *On Mason's marks in particular, and Mediaeval Craftsman's marks in general*. He was prominent in all affairs of Selby, and had served as president of a considerable number of associations connected with the town as well as being a Justice of the Peace for the West Riding. In his younger days he was a strong swimmer—he swam the Humber while at Hull—and took a great delight in all kinds of sport. It was a pleasure to be in his company as he had so many interests and, moreover, enjoyed a good story—either in the telling or in the hearing; "one of Cheesman's" used often to go the rounds of a foray. His death removes from our circle one who was sincerely respected by all. To the younger mycologists he was representative of a type that seems in danger of being swamped by the greatly increased numbers of professional workers. In these days when there are so many pleasures in which business men may obtain their necessary relaxation it should be the aim of the professional worker to encourage business-men naturalists and working-men naturalists to continue to share those of our labours and our delights that their occupation allows.

The death of William Norwood Cheesman takes from our ranks in the year in which he occupied the premier position a very fine type of English gentleman: generous, kind, earnest, and one who had the interests of his country at heart.

It is a pleasing thought that his name will ever be associated with the Society in connection with the Cheesman Fund.

## THE TINTERN FORAY.

April 24th to 28th, 1925.

By E. M. Wakefield, M.A., F.L.S.

THE Spring Foray was held at Tintern from April 24th to April 28th, with headquarters at the Beaufort Arms Hotel. About fifteen members assembled during the evening of the 24th, some having already been out and gathered a few species, chiefly of micro-fungi.

On Saturday morning a start was made at 10 a.m. for the woods lying north of Tintern. It was soon apparent that the larger fungi were extremely scarce, probably owing to the prevailing cold, dry winds, and that the list would have to be made up chiefly of micro-forms. Along the hedge bank of a narrow lane leading upwards behind the village were found several Rusts, including *Puccinia tumida* on *Conopodium*, *Milesina Scolopendrii* on the hart's-tongue fern, and *Milesina Polystichi* on *Polystichum angulare*. The two latter species, and also *M. Kriegeriana* on the male fern, were found in some abundance throughout the foray, and it is interesting to record that *M. Scolopendrii* and *M. Kriegeriana* had also been found to be abundant in South Devon just a week earlier. In a coppice near the top of the hill a very fine specimen of *Dermatea Cerasi* was obtained on dead branches of *Prunus Avium*.

On Sunday morning some of the members spent a short time in a wood beside the railway on the Gloucestershire side of the River Wye. This wood was very damp in the lower part, near the river, and consequently some of the larger Discomycetes were found here, which did not occur elsewhere during the foray. *Mitrophora hybrida* was soon noticed, and Miss Brett later picked up a fine specimen of *Disciotis venosa*. Both *Ciliaria scutellata* and *C. trechispora* were also found. Dead twigs, of willow and ash chiefly, yielded various Pyrenomycetes, and also *Propolis faginea*.

In the afternoon the whole party walked to Wyndcliff, obtaining on the way two interesting Discomycetes growing on the dark basal part of dead stems of *Pteris*, namely *Micropodia pteridina*, and a pale yellowish form somewhat resembling *Helotium cyathoides*, which was subsequently identified by Mr W. D. Buckley as probably *Dasyscypha caulicola* (Fr.) Boud., about which little seems to be known. Dr Malcolm Wilson secured *Synchytrium Mercurialis* and later *S. Taraxaci*. On the descent from the Wyndcliff some very fine specimens of *Daldinia concentrica* were noted.



On Monday morning the woods on Chapel Hill were visited. The ground here was very dry and little of interest was obtained. In a small marshy spot at the bottom of the hill some dead giant *Heracleums* were found, whose stems were covered with fine fructifications of *Heterosphaeria Patella*, unfortunately however with the asci as yet immature. One small dead twig, unidentified, yielded a few perithecia with the characteristic spores of *Leptospora caudata* Fuck.

The party returned home for lunch and in the afternoon another visit was paid to some of the ground covered on Saturday, which so far had proved most productive, the return being made by way of Barbados Hill.

Here Miss Roper found a plant or two of *Jasione montana* showing blackish pustules on the leaves, which to the naked eye appeared like one of the Rusts. Microscopic examination showed however that the fungus resembled the early stage of certain Discomycetes. Unfortunately it was quite immature, but the species if found again may prove to be *Pyrenopeziza Jasiones* Romell, which would be a new record for this country.

*Taphridium umbelliferarum* occurred scantily in a hedge, and towards the end of the walk a mass of dead stems of broom yielded *Nectria sanguinea*, *N. mammoidea* and *Volutella ciliata*, all in considerable abundance. Ivy leaves which were brought back with *Phyllosticta hedericola* and *Septoria Hederae*, later produced also *Vermicularia trichella* and *Mycosphaerella hedericola*.

A short business meeting was held on the Monday evening, with Mr Pearson in the chair. The President, Mr Cheesman, sent apologies for his absence through ill-health, and best wishes for the success of the meeting. A letter was read from Dr Durham, President of the Woolhope Club, expressing a hope that the Society would find it possible to hold a foray in conjunction with the Woolhope Club, some time in the near future. As the 1925 Foray was already fixed for Dublin, and an alternative foray was considered not practicable, it was decided that the General Secretary should be asked to write to Dr Durham suggesting that the Autumn Foray in 1926 might be held at Hereford.

The locale of the next Spring Foray was also considered, and after some discussion members voted in favour of Arundel if possible, or if not some other convenient spot in the South Eastern district.

Two new members, Dr O. F. Burger of Gainesville, Florida, and Mr W. F. Hanna were elected, and the meeting closed with hearty votes of thanks to the Commissioners of Woods and Forests and to Mr Hastings Clay, for permission to work over their lands.

For assistance in compiling the subjoined list the Secretary is indebted to all the members present.

*Complete List of Fungi gathered during the Foray\*.*

**HYMENOMYCETES.**

- Armillaria mellea* (Vahl.) Fr. (rhizomorphs only).  
*Omphalia Swartzii* Fr.  
*Panus stipticus* (Bull.) Fr.  
*Lenzites betulina* (Linn.) Fr.  
*Entoloma clypeatum* (Linn.) Fr.  
*Claudopus variabilis* (Pers.) W. G. Sm.  
*Galera tenera* (Schaeff.) Fr., *hypnorum* (Schränk.) Fr.  
*Tubaria furfuracea* (Pers.) W. G. Sm.  
*Hypholoma sublateritium* (Schaeff.) Fr., *fasciculare* (Huds.) Fr.  
*Coprinus micaceus* (Bull.) Fr.  
*Panaeolus sphinctrinus* Fr.  
*Psathyrella atomata* Fr.  
*Fomes annosus* Fr.  
*Polystictus versicolor* (Linn.) Fr.  
*Daedalea quercina* (Linn.) Fr.  
*Irpex obliquus* (Schrad.) Fr.  
*Radulum orbiculare* Fr.  
*Odontia bicolor* (A. & S.) Bres., *farinacea* (Pers.) Quél.  
*Mycoleptodon fimbriatum* (Pers.) Bourd. and Galz.  
*Stereum hirsutum* (Willd.) Fr.  
*Hymenochaete corrugata* (Fr.) Lév., *tabacina* (Sow.) Lév.  
*Corticium laeve* (Pers.) Fr., *niveo-cremum* v. Hoehn. & Litsch., *Sambuci* (Pers.) Fr., *Pearsonii* Bourd., *comedens* (Nees) Fr., *porosum* Berk. & Curt., *praetermissum* (Karst.) Bres.  
*Peniophora glebulosa* (Fr.) Bres., *pallidula* Bres., *longispora* (Pat.) v. Hoehn. & Litsch., *cremea* Bres., *gigantea* (Fr.) Mass., *incarnata* (Pers.) Cooke, *cinerea* (Fr.) Cooke, *laevigata* (Fr.) Mass.  
*Auricularia Auricula-Judae* (Linn.) Schroet.  
*Exidia glandulosa* (Bull.) Fr., *nucleata* (Schwein.) Rea.

**UREDINEAE.**

- Uromyces Ficariae* (Schum.) Lév., *Valerianae* (Schum.) Fuck., *Scillarum* (Grev.) Wint., *Dactylidis* Otth., *Poa* Rabenh.  
*Puccinia fusca* (Relh.) Wint., *Anemones* Pers., *Violae* (Schum.) DC., *Lychnidearum* Link., *Umbilici* Guep., *Aegopodii* (Schum.) Mart., *tumida* Grev., *albescens* Grev., *Adoxae* Hedw. fil., *Lampsanae* (Schultz.) Fuck., *Taraxaci* Plowr., *obscura* Schroet., *Caricis* (Schum.) Rebert., *holcina* Erikss., *dispersa* (sens. lat.) Erikss. & Henn. on *Aira caespitosa*, *graminis* Pers. on *Agropyrum repens*, *oblongata* (Link) Wint. on *Luzula maxima*.  
*Phragmidium violaceum* (Schultz.) Wint., *Fragariae* (DC.) Wint.  
*Melampsora Rostropii* Wagn. (= *Caeoma Mercurialis* (Mart.) Link).  
*Melampsorium betulinum* (Pers.) Kleb.  
*Milesina Scolopendrii* (Fuck.) Jaap, *Kriegeriana* P. Magn., *Polystichi* (Wineland) Grove.

**PYRENOMYCETES.**

- Sphaerotheca tomentosa* Otth. on *Euphorbia amygdaloides*.  
*Erysiphe Polygoni* DC. on *Heracleum*, *graminis* DC.  
*Nectria cinnabarina* (Tode) Fr., *mammoidea* Phil. & Plowr., *sanguinea* (Sibth.) Fr., *episphaeria* (Tode) Fr.  
*Rosellinia aquila* (Fr.) de Not.

\* The localities for Monmouthshire are not recorded separately, as all were within a few miles of Tintern. A separate list is appended of species collected on the opposite side of the Wye, in Gloucestershire.

*Stigmata Robertiani* Fr.  
*Mycosphaerella hedericola* (Desm.) Lindau, *maculiformis* (Pers.) Schroet.  
*Venturia Rumicis* (Desm.) Wint.  
*Leptosphaeria acuta* (Moug. & Nestl.) Karst.  
*Leptospora caudata* Fuck.  
*Hypospila Pustula* (Pers.) Karst.  
*Didymella Salicis* Grove.  
*Diaporthe leiphaemia* (Fr.) Sacc.  
*Gnomonia cerastis* (Riess) Ces. & de Not.  
*Eutypa lata* (Pers.) Tul., *flavo-virescens* (Hoffm.) Sacc.  
*Valsa decorticans* Fr.  
*Diatrypella quercina* (Pers.) Nke.  
*Diatrype Stigma* (Hoffm.) de Not., *disciformis* (Hoffm.) Fr.  
*Hypoxylon multifforme* Fr., *coccineum* Bull., *fuscum* Fr.  
*Xylaria Hypoxylon* (L.) Grev.  
*Daldinia concentrica* (Bolt.) Ces. & de Not.  
*Ustilina vulgaris* Tul. (Conidial stage).  
*Phyllachora graminis* (Pers.) Fuck.

## HYSTERIACEAE.

*Rhopographus Pteridis* (Sow.) Wint.  
*Dichaena faginea* Fr., *quercina* Fr.  
*Gloniopsis curvata* Sacc.

## DISCOMYCETES.

*Sarcoscypha coccinea* (Jacq.) Fr.  
*Ciliaria scutellata* (Linn.) Quél.  
*Ombrophila clavus* (A. & S.) Cooke.  
*Coryne sarcoides* (Jacq.) Tul.  
*Bulgaria inquinans* (Pers.) Fr.  
*Calloria fusarioides* (Berk.) Fr.  
*Orbilbia xanthostigma* Fr.  
*Chlorosplenium aeruginosum* (Oeder) de Not. (Mycelium only).  
*Helotium fructigenum* (Bull.) Fuck.  
*Dasyphypha virginea* (Batsch) Fr., *caulicola* Fr.? (on *Pteris* stems).  
*Trichoscypha calycina* (Schum.) Boud.  
*Arachnopeziza aurelia* (Pers.) Fuck.  
*Micropodia pteridina* (Karst.) Boud.  
*Mollisia cinerea* (Batsch) Karst.  
*Heterosphaeria Patella* (Tode) Grev.  
*Dermatea Cerasi* (Pers.) de Not.  
*Pseudopeziza Trifolii* (Biv.-Bern) Fuck., *Ranunculi* (Wallr.) Fuck., *repanda* (Fr.) Karst.  
*Stegia Illicis* Fr.  
*Colpoma quercinum* (Pers.) Wallr.  
*Rhytisma acerinum* (Pers.) Fr.

## PHYCOMYCETES.

*Synchytrium Taraxaci* de By. & Woron., *Mercurialis* (Lib.) Fuck.

## PROTOMYCETACEAE.

*Taphridium umbelliferarum* (Rostr.) Juel.

## SPHAEROPSIDACEAE.

*Phyllosticta hedericola* Dur. & Mont.  
*Phoma herbarum* West. (on dead stems of *Verbascum*).  
*Septoria Hederae* Desm.

## MELANCONIACEAE.

*Marssonina Potentillae* (Desm.) P. Magn.

**HYPHOMYCETES.**

Ovularia obliqua (Cooke) Oud.  
Botrytis cinerea (Pers.) Fr.  
Scolecotrichum graminis Fuck.  
Volutella ciliata (A. & S.) Fr.  
Vermicularia trichella Fr.

*Species found on Gloucestershire side of River Wye.*

Panus stipticus (Bull.) Fr.  
Polystictus versicolor (Linn.) Fr.  
Merulius corium (Pers.) Fr.  
Trametes mollis (Sommerf.) Fr.  
Corticium caeruleum (Schrad.) Fr., comedens (Nees) Fr.  
Peniophora caesia (Bres.) Bourd. & Galz., cinerea (Fr.) Cooke.  
Hymenochaete rubiginosa (Dicks.) Lév., corrugata (Fr.) Lév.  
Uromyces Scillarum (Grev.) Wint.  
Puccinia Violae (Schum.) DC., Hieracii Mart., obscura Schroet., holcina Erikss.  
Phragmidium violaceum (Schultz.) Wint., Fragariae (DC.) Wint.  
Melampsora Rostrupii Wagn. (Aecidium on *Mercurialis*).  
Milesina Polystichi (Wineland) Grove.  
Stigmatia Robertiani Fr.  
Mycosphaerella maculiformis (Pers.) Schroet.  
Hypospila Pustula (Pers.) Karst.  
Didymella Salicis Grove.  
Cryptosphaeria eunomia (Fr.) Fuck.  
Diatrype disciformis (Hoffm.) Fr.  
Eutypa lata (Pers.) Tul.  
Hypoxylon fuscum Fr., rubiginosum (Pers.) Fr.  
Xylaria Hypoxylon (Linn.) Grev.  
Phyllachora graminis (Pers.) Fuck.  
Rhopoglyphus Pteridis (Sow.) Wint.  
Gloniopsis curvata Sacc.  
Mitrophora hybrida (Sow.) Boud.  
Disciotis venosa (Pers.) Boud.  
Sarcoscypha coccinea (Jacq.) Fr.  
Ciliaria scutellata (Linn.) Quél., trechispora (B. & Br.) Boud.  
Mollisia cinerea (Batsch) Karst.  
Pseudopeziza repanda (Fr.) Karst.  
Propolis faginea (Schrad.) Karst.  
Stegia Ilicis Fr.  
Phyllosticta hedericola Dur. & Mont.  
Septoria Hederae Desm.

**LICHENS OF THE TINTERN FORAY.**

*By H. H. Knight, M.A.*

THE rocks in the Tintern district were similar to those met with at the Spring Foray at Matlock last year. In both districts Carboniferous Limestone rocks occur, but instead of the Millstone Grit of Derbyshire we had Old Red Sandstone rocks. The Wye Valley at Tintern however is well wooded, and consequently calcareous lichens were less plentiful than in the exposed valleys of Dovedale and Millers Dale. The most interesting find was *Opegrapha paraxanthodes*, a lichen of shady calcareous rocks.

Several of the lichens in this list were found on sandstone walls; these compared favourably with the grit walls in the Matlock district.

Tree lichens were more numerous than in Derbyshire, though only some of the common species were met with.

The parasitic fungus *Ticothecium rimosicolum* Arn. was found growing on *Rhizocarpon petraeum*.

In the *Journal of Botany*, May 1925, Dr Watson has described under the name *Clathroporina calcarea* a lichen found at the foray last year in Dovedale and Millers Dale.

A few lichens in this list (indicated by the letter G.) were found on the left bank of the River Wye in Gloucestershire.

- |  |  |
|--|--|
| Placynthium nigrum S. F. Gray                | Diploschistes scruposus Norm.                    |
| Collema cheileum Ach.                        | Cladonia sylvatica Hoffm.                        |
| C. granuliferum Nyl.                         | C. pyxidata Hoffm.                               |
| Leptogium lacerum S. F. Gray                 | C. fimbriata Fr.                                 |
| Peltigera canina Willd.                      | C. furcata Schrad.                               |
| P. horizontalis Hoffm.                       | Coenogonium ebeneum A. L. Sm.                    |
| Parmelia physodes Ach.                       | Gyalecta cupularis Schaer.                       |
| P. caperata Ach.                             | Lecidea lucida Ach.                              |
| P. sulcata Tayl.                             | L. uliginosa Ach.                                |
| P. dubia Tayl.                               | L. parasema Ach.                                 |
| P. fuliginosa Nyl. and var. laetevirens Nyl. | L. contigua Fr.                                  |
| Evernia prunastri Ach.                       | L. solediza Nyl.                                 |
| Ramalina farinacea Ach.                      | L. confluens Ach.                                |
| Usnea florida Web. var. hirta Ach.           | L. lithophila Ach.                               |
| Xanthoria parietina Th. Fr.                  | L. fuscoatra Ach., G.                            |
| X. polycarpa Oliv.                           | Biatorina cyrtella Th. Fr., G.                   |
| Placidium murorum DC.                        | Bilimbia aromatica Jatta                         |
| P. aurantiacum Anzi var. flavovirens Anzi    | B. sabuletorum Br. & Rostr.                      |
| Physcia hispida Tuckerm.                     | Buellia canescens de Not.                        |
| Lecanora subfusca Ach.                       | B. myriocarpa Mudd                               |
| L. campestris B. de Lesd.                    | Rhizocarpon petraeum Massal.                     |
| L. atra Ach.                                 | Arthonia lurida Ach. var. spadicea Nyl.          |
| L. Hageni Ach.                               | A. radiata Ach.                                  |
| L. varia Ach.                                | Opegrapha paraxanthodes Nyl.                     |
| L. polytropa Schaer.                         | Graphis elegans Ach., and f. stellata Leight.    |
| L. parella Ach.                              | Graphis scripta Ach., and var. pulverulenta Ach. |
| Acarospora fuscata Th. Fr.                   | Verrucaria nigrescens Pers.                      |
| Lecania syringea Th. Fr., G.                 | Arthopyrenia fallax Arn.                         |
| Pertusaria faginea Leight.                   | Porina carpinea A. Zahlbr.                       |
| P. pertusa Dal. Tor. and Sarnth.             | P. chlorotica Wainio                             |
| P. leioplaca Schaer.                         |  |
| Phlyctis argena Koerb.                       |  |

## THE DUBLIN FORAY.

September 21st to 26th, 1925.

By E. M. Wakefield, M.A., F.L.S.

THE twenty-ninth Autumn Foray and Annual General Meeting of the Society was held at Dublin from September 21st to September 26th, at the invitation of a committee of well-known Irish botanists.

Most of the party were very comfortably housed at the Standard Hotel, but meetings were held at the Royal College of Surgeons, where Professor J. Alfred Scott had placed at our disposal not only a room for meetings and the reading of papers, but also a laboratory for work and the exhibition of specimens. In addition, through the kind offices of Miss Knowles and Mr Gorman, books from the libraries of the Natural History Museum and of the College of Science were available for use, so that facilities for the naming of material on the spot were better than usual. This, and the inclusion in the party of several members interested chiefly in parasitic fungi, perhaps in part accounts for the large number of Fungi Imperfecti recorded, species of *Ramularia* in particular being unusually numerous.

The majority of the party, including about twenty members who had crossed the Irish Channel, assembled on Monday evening, September 21st, but various members had taken advantage of an excursion ticket which brought them a few days earlier, and had already carried out some preliminary skirmishes. Mr Muskett and Mr Carrothers brought from the neighbourhood of Belfast a number of the larger fungi, which made an interesting first exhibit.

The first "official" expedition was a whole day on Tuesday in the beautiful Powerscourt Demesne, a start being made from the Hotel at 9.30 a.m. Heavy rain was experienced in the middle of the day, but there was compensation in seeing the fine waterfall at its best. In the afternoon the sun came out and wet feet and dripping waterproofs were soon forgotten, particularly over a substantial Irish tea provided at the Lodge at the end of the day.

The Powerscourt estate yielded some of the best finds of the week amongst the larger fungi, for instance *Lepiota lenticularis*, *Pleurotus geogenius*, *Leptonia chalybea*, *Psaliota haemorrhoidaria* (found in abundance in an open grassy spot), and various species of *Hygrophorus* and *Inocybe*.

*Cortinarii* were rare, as all through the week, only a few of the smaller and more common species being found. The impression gained was that the "run" of Agarics had not yet

properly developed, and from reports received since as to their unkind behaviour in coming up in abundance after we had left, this appears to have been the case.

On Tuesday evening at 8.45, the Annual General Meeting was held. In the unfortunate absence of the President owing to illness, the chair was taken by the Vice-President, Mr J. Ramsbottom. Dr G. H. Pethybridge was unanimously elected as President for 1926, with Dr E. J. Butler as Vice-President. In place of Mr Dowson and Mr Sharpe, the retiring members of Council, Mr W. Buddin and Mr F. G. Gould were elected. The other officers remain as before except that Mr Pearson resigned the office of Foray Secretary\*. Miss Wakefield was asked to represent the Society at the British Association meeting at Oxford in 1926. For the 1926 Autumn Foray several invitations had been received. After some discussion it was decided to arrange a joint foray with the Woolhope Club at Hereford in 1926, and if possible a foray with the Scottish Cryptogamic Society in 1927. Finally the meeting heard with much pleasure and gratitude that the President, Mr W. N. Cheesman, had presented to the Society the sum of one hundred guineas, to be invested, and the interest to be used as the Council should think best.

On the Wednesday visits to the Universities, Museum, and Glasnevin Botanic Gardens filled up the morning, and these were followed by lunch by kind invitation with the Dublin Rotary Club, when Mr Ramsbottom gave a short address on "Mycology and Human Affairs." After lunch an expedition was made to Howth, where various additions to the list of micro-fungi were made. Members who had been to Glasnevin brought back the interesting *Rhizosphaera Kalkhoffii* Bub., only recently recorded in Scotland on the same host, *Picea pungens*.

In the evening, in the absence of the usual Presidential address, Mr Ramsbottom gave a talk on "The Ecology of Fungi."

On Thursday a start was made at 11.30 a.m. for Carton Demesne. Here the ground first covered was a mixed plantation of Conifers and Dicotyledonous trees, traversed by wide grassy rides, which provided good hunting for both large and small fungi. *Hebeloma fastibile* was fairly common in the rides, and evidence of the presence of Conifers was provided by such species as *Boletus viscidus*, *B. elegans*, and *Tremellodon gelatinosum*. After leaving this plantation the party worked through

\* At a meeting of the Council held November 6th, it was decided not to appoint a separate Foray Secretary, but that the Secretary should carry out the general arrangements, and that where possible a temporary local secretary should be nominated to undertake the necessary local details.

Bog Wood towards the lake, finally meeting for tea at the curious "Shell Cottage." One of the kind thoughts of our guides which was much appreciated was the provision of small photographic reproductions of the Ordnance maps of the ground worked each day, whereby even the most incorrigible strays were enabled to turn up for tea and the return home at the hour specified. *Fomes annosus* was found on ash, on which host it has also been seen at Kew. In addition to the larger fungi, numerous micro-fungi were recorded on this day, including various species of *Ramularia*, *Cercospora Opuli* on *Viburnum Opulus*, *Microsphaeria Euonymi* showing perithecia, *Puccinia Buxi*, and *Milesina Scolopendrii*.

In the evening the members were entertained at dinner at University College by the Dublin Natural History Society.

On Friday Kilruddery, the property of Lord Ardee, was visited. The members were received by Lady Ardee and first shown over the house, and afterwards her agent Mr Le Fanu acted as guide through the grounds. Unfortunately rain was again experienced, but in spite of it a very fair bag was made, notably of the smaller forms.

In the evening a number of our Irish friends joined us at dinner at the hotel, and afterwards attended the final meeting, when comments on the finds of the week were made by Miss Lister, Miss Lorrain Smith, Miss Wakefield, Mr Pearson and Mr Ramsbottom. In conclusion the usual votes of thanks to landowners, etc., were passed, and particularly hearty thanks were expressed for the great kindness we had experienced in every way in Dublin.

The following list includes not only the species gathered during the official period of the Foray, but also numerous finds made by early arrivals. Thus Dr Butler had collected various fungi at Malahide, and Miss Lister brought in *Polyporus hispidus*, *P. squamosus*, and *Ganoderma applanatum* from near Dublin. The records from the Scalp and Enniskerry were made by some of the members who had arrived on Saturday and were taken a most enjoyable expedition on the Sunday. Mr Cotton stayed after the end of the Foray and brought back a few additional records from Avondale.

The ground covered includes more than one county. Thus while the Scalp, Malahide and Howth are in Co. Dublin, Powerscourt, Enniskerry, and Kilruddery are in Co. Wicklow, and Carton is in Co. Kildare.

For assistance in compiling the list the Secretary is indebted to all the members present, and more particularly to Mr Ramsbottom, Dr Butler, Mr Pearson, and Mr Buddin.

The species marked with an asterisk are not included in



either Adams and Pethybridge, *Census Catalogue of Irish Fungi* (1910) or Rea and Hawley's list of fungi in the Clare Island Survey reports (1912). The further mark † indicates that these species are apparently new to the British Flora.

*Complete List of Species gathered during the Foray.*

S. = the Scalp; E. = Enniskerry; P. = Powerscourt; H. = Howth;  
C. = Carton Demesne; K. = Kilruddery.

**HYMENOMYCETES.**

- Amanita phalloides* (Vaill.) Fr., S., P., K., *mappa* (Batsch) Fr., S., P., *pantherina* (DC.) Fr., P.  
*Amanitopsis vaginata* (Bull.) Roze, H., *stragulata* (Fr.) Roze, C.  
*Armillaria mellea* (Vahl) Fr., P., K., (rhizomorphs only) E., H., C., K.  
*Lepiota procera* (Scop.) Fr., P., *rhacodes* (Vitt.) Fr., P., *acutesquamosa* (Weinm.) Fr., C., *amaianthina* (Scop.) Fr., K., *carcharias* (Pers.) Fr., P., *lenticularis* (Lasch) Cooke\*, P.  
*Tricholoma rutilans* (Schaeff.) Fr., H., C., K., *terreum* (Schaeff.) Fr., P., *argyraceum* (Bull.) Fr., P., *cuneifolium* Fr., P., *saponaceum* Fr., P., *sulphureum* (Bull.) Fr., K., *album* (Schaeff.) Fr., P., *nudum* (Bull.) Fr., P., *cinerascens* (Bull. non Fr.) Quél., P., K., *melaleucum* (Pers.) Fr., P., C., K.  
*Clitocybe aurantiaca* (Wulf.) Studer, S., P., H., K., *cerussata* Fr., C., *flaccida* (Sow.) Fr., P., *fragrans* (Sow.) Fr., P., C.  
*Laccaria laccata* (Scop.) B. & Br., P., H., C.  
*Collybia radicata* (Reh.) Berk., H., K., *maculata* (A. & S.) Fr., P., *cirrhatta* (Schum.) Fr., K., *ambusta* Fr., P., *atrata* Fr., P.  
*Mycena lineata* (Bull.) Fr., K., *rugosa* Fr., C., K., *galericulata* (Scop.) Fr., P., K., *polygramma* (Bull.) Fr., H., K., *inclinata* Fr., P., C., K., *alcalina* Fr., P., *ammoniaci* Fr., P., C., *metata* Fr., K., *vitis* Fr., P., K., *haematopus* (Pers.) Fr., P., *sanguinolenta* (A. & S.) Fr., S., P., H., K., *galopus* (Pers.) Fr., P., H., C., K., var. *alba* Fl. Dan., C., var. *nigra* Fl. Dan., P., *pelliculosa* Fr., P., *rorida* Fr., C., *stylobates* (Pers.) Fr., C., *corticola* (Schum.) Fr., P.  
*Omphalia pyxidata* (Bull.) Fr., H., S., *umbellifera* (Linn.) Fr., S., *fibula* (Bull.) Fr., P., C., K.  
*Pleurotus ostreatus* (Jacq.) Fr., H., *geogenius* (DC.) Fr., P., *applicatus* (Batsch) Berk., P.  
*Hygrophorus limacinus* Fr., C., K., *pratensis* (Pers.) Fr., P., K., *virginus* (Wulf.) Fr., P., *niveus* (Scop.) Fr., P., *subradiatus* (Schum.) Fr., var. *lacmus* Fr., P., *coccineus* (Schaeff.) Fr., P., *miniatus* Fr., P., *puniceus* Fr., P., *obrusseus* Fr., P., K., *conicus* (Scop.) Fr., P. (yellow form), C., K., *chlorophanus* Fr., P., *psittacinus* (Schaeff.) Fr., P.  
*Lactarius turpis* (Weinm.) Fr., E., H., *pubescens* Fr., C., *blennius* Fr., K., *circellatus* Fr., H., *vellereus* Fr., C., *deliciosus* (Linn.) Fr., P., *galidus* (Pers.) Fr., P., *quietus* Fr., S., P., K., *vietus* Fr., H., *glyciosmus* Fr., H., *serifluus* (DC.) Fr., E., *mitissimus* Fr., P., *subdulcis* (Pers.) Fr., P.  
*Russula chloroides* (Krombh.) Bres., P., K., *nigricans* (Bull.) Fr., E., K., *adusta* (Pers.) Fr., P., *lepida* Fr., K., *cyanoxantha* (Schaeff.) Fr., S., P., *furcata* (Pers.) Fr., S., *ochroleuca* (Pers.) Fr., S., H., C., K., *fellea* Fr., P., H., *sanguinea* (Bull.) Fr., P., *drimeia* Cooke, S., P., *fragilis* (Pers.) Fr., P., H., K., var. *fallax* (Schaeff.) Mass., S., P., C., K., *caerulea* Cooke, S., *lutea* (Huds.) Fr., P., var. *armeniaca* (Cooke) Rea, P., *atropurpurea* (Krombh.) Maire, K.  
*Cantharellus cibarius* Fr., P.  
*Marasmius peronatus* (Bolt.) Fr., P., H., K., *oreades* (Bolt.) Fr., E., *erythropus* (Pers.) Fr., K., *hariolorum* (DC.) Quél., P., C., K., *dryophilus* (Bull.) Karst., P., H., K., *ramealis* (Bull.) Fr., H.  
*Androsaceus rotula* (Scop.) Pat., P., H., C., K., *androsaceus* (Linn.) Pat., P., H.

- Panus torulosus* (Pers.) Fr., P., K., *stipticus* (Bull.) Fr., P.  
*Volvaria parvula* (Weinm.) Fr., P.  
*Pluteus cervinus* (Schaeff.) Fr., P., C., K., *nanus* (Pers.) Fr., var. *lutescens* Fr., C., K.  
*Entoloma sericeum* (Bull.) Fr., P., C., K.  
*Leptonia chalybaea* (Pers.) Fr., P.  
*Nolanea pascua* (Pers.) Fr., S., *papillata* Bres., P.  
*Clitopilus prunulus* (Schaeff.) Fr., K.  
*Paxillus involutus* (Batsch) Fr., P., H., K., *panuoides* Fr., P.  
*Pholiota erebia* Fr., P., C., *squarrosa* (Müll.) Fr., H., K., *adiposa* Fr., K., *mutabilis* (Schaeff.) Fr., P., K., *marginata* (Batsch) Fr., K.  
*Inocybe pyriodora* (Pers.) Fr., P., *rimosa* (Bull.) Fr., P., C., *tomentosa* (Jungh.) Quél., P., *geophylla* (Sow.) Fr., C., K., var. *lilacina* Fr., P., C., K., *Godeyi* Gill., P., H., *obscura* (Pers.) Fr., C., *flocculosa* Berk., P., *cinnamomea* Fr., P., *calamistrata* Fr., S., *fastigiata* (Schaeff.) Fr., K.  
*Astrosporina proximella* (Karst.) Rea\*, P., *praetervisa* (Quél.) Schroet., C., *calospora* (Quél.) Rea\*, P.  
*Hebeloma fastibile* Fr., C., *crustuliniforme* (Bull.) Fr., P., H., C., var. *minus* Cooke\*, C.  
*Flammula ochrochlora* Fr., P., *carbonaria* Fr., P., K.  
*Naucoria cucumis* (Pers.) Fr., P., *melinoides* Fr., P., *escharoides* Fr., C., K.  
*Galera tenera* (Schaeff.) Fr., H., C., *hypnorum* (Schränk) Fr., H.  
*Tubaria furfuracea* (Pers.) W. G. Sm., P., C., K., *pellucida* (Bull.) Fr., P.  
*Crepidotus mollis* (Schaeff.) Fr., P., K.  
*Cortinarius* (*Myxaciium*) *elator* Fr., P.  
*Cortinarius* (*Telamonia*) *hinnuleus* (Sow.) Fr., P., C., K., *brunneus* (Pers.) Fr., P., *flexipes* Fr., C., *rigidus* (Scop.) Fr., P., *paleaceus* (Weinm.) Fr., C., K.  
*Psaliota campestris* (Linn.) Fr., P., C., K., *haemorrhoidaria* Kalchbr., P.  
*Stropharia semiglobata* (Batsch) Fr., P., H., K., *aeruginosa* (Curt.) Fr., K.  
*Hypholoma fasciculare* (Huds.) Fr., S., H., C., K., *sublateritium* (Schaeff.) Fr., C., *pyrotrichum* (Holmsk.) Fr., P., *pilulaeforme* (Bull.) Fr., P., K., *hydrophilum* (Bull.) Fr., P., K.  
*Psilocybe sarcocephala* Fr., P., *uda* (Pers.) Fr., P., *semilanceata* Fr., H., C., K., *spadicea* Fr., K.  
*Psathyra corrugis* (Pers.) Fr., P., K., *fibrillosa* (Pers.) Fr., P., C., K.  
*Psathyrella gracilis* Fr., P., H., C., K., *atomata* Fr., C.  
*Panaeolus sphinctrinus* Fr., H., K., *campanulatus* (Linn.) Fr., P., C., K.  
*Anellaria separata* (Linn.) Karst., C.  
*Bolbitis titubans* (Bull.) Fr., C., K.  
*Coprinus comatus* (Fl. Dan.) Fr., P., H., C., K., *sterquilinus* Fr., P., C., *atramentarius* (Bull.) Fr., K., *niveus* (Pers.) Fr., H., *micaceus* (Bull.) Fr., H., K., *Hendersonii* Berk., C., K., *domesticus* (Pers.) Fr., K., *lagopus* Fr., C., K., *plicatilis* (Curt.) Fr., P., H., C., K.  
*Gomphidium viscidum* (Linn.) Fr., P., C., K.  
*Lenzites betulina* (Linn.) Fr., P.  
*Boletus elegans* (Schum.) Fr., P., C., *viscidus* (Linn.) Fr., C., *granulatus* (Linn.) Fr., P., *badius* Fr., P., *chrysenteron* (Bull.) Fr., S., P., K., *subtomentosus* (Linn.) Fr., S., *edulis* (Bull.) Fr., E., *scaber* (Bull.) Fr., E.  
*Fistulina hepatica* (Huds.) Fr., C.  
*Polyporus varius* Fr., P., *squamosus* (Huds.) Fr., P., H., C., *sulphureus* (Bull.) Fr., P., K., *giganteus* (Pers.) Fr., C., K., *betulinus* (Bull.) Fr., P., H., *dryadeus* (Pers.) Fr., P., *hispidus* (Bull.) Fr., K., *adiposus* B. & Br., H., *adustus* (Willd.) Fr., P., *caesius* (Schräd.) Fr., P.  
*Fomes annosus* Fr., P., C., K. (on Ash), *connatus* Fr., K. (on Elm).  
*Ganoderma applanatum* (Pers.) Pat., H., C., K.  
*Polystictus versicolor* (Linn.) Fr., E., H., K., *abietinus* (Dicks.) Fr., K.  
*Ptychogaster albus* Corda, K.  
*Daedalea biennis* (Bull.) Quél., P., *quercina* (Linn.) Fr., P.  
*Merulius corium* (Pers.) Fr., C., K., *lacrymans* (Wulf.) Fr., Dublin, *fugax* Fr., S.  
*Hydnum repandum* (Linn.) Fr., E.

- Caldesiella crinalis* (Fr.) Bourd. & Galz., *P.*  
*Radulum orbiculare* Fr., *H.*  
*Acia uda* (Fr.) Bourd. & Galz., *C.*  
*Grandinia granulosa* Fr., *Malahide*.  
*Irpex obliquus* (Schrad.) Fr., *P.*, *H.*, *C.*, *K.*  
*Phlebia merismoides* Fr., *C.*  
*Hypochnus ferrugineus* (Pers.) Fr., *K.*, *fuscus* (Pers.) Fr., *P.*  
*Stereum spadiceum* Fr., *P.*, *rugosum* (Pers.) Fr., *P.*, *H.*, *hirsutum* (Willd.) Fr., *P.*, *C.*, *K.*, *purpureum* (Pers.) Fr., *P.*, *C.*  
*Hymenochaete rubiginosa* (Dicks.) Lév., *P.*  
*Corticium laeve* (Pers.) Fr., *P.*, *H.*, *C.*, *K.*, *arachnoideum* Berk., *S.*, *Sambuci* (Pers.) Fr., *Malahide*, *C.*, *K.*, *confluens* Fr., *Malahide*, *P.*, *comedens* (Nees) Fr., *P.*, *porosum* Berk. & Curt., *K.*, *praetermissum* (Karst.) Bres., *P.*  
*Peniophora Aegerita* v. Hoehn. & Litsch., *K.*, *pallidula* Bres., *E.*, *longispora* (Pat.) v. Hoehn. & Litsch., *E.*, *setigera* (Fr.) Bres., *C.*, *cinerea* (Fr.) Cooke, *E.*, *H.*, *C.*, *K.*, *quercina* (Pers.) Cooke, *P.*, *C.*, *K.*  
*Coniophora puteana* (Schum.) Fr., *Dublin*.  
*Clavaria inaequalis* (Müll.) Fr., *P.*, *K.*, *vermicularis* Fr., *P.*, *fumosa* (Pers.) Fr., *P.*  
*Pistillaria quisquiliaris* Fr., *S.*, *P.*, *puberula* Berk., *P.*  
*Auricularia auricula-Judae* (Linn.) Schroet., *P.*, *H.*, *C.*, *K.*  
*Tremella mesenterica* (Retz.) Fr., *P.*, *K.*  
*Exidia glandulosa* (Bull.) Fr., *H.*, *C.*, *nucleata* (Schw.) Rea\*, *C.*, *Thurettiana* (Lév.) Fr., *P.*, *C.*  
*Tremellodon gelatinosum* (Scop.) Pers., *P.*, *C.*  
*Dacryomyces deliquescens* (Bull.) Duby, *S.*, *P.*, *K.*  
*Calocera viscosa* (Pers.) Fr., *E.*, *K.*, *cornea* (Batsch) Fr., *P.*, *stricta* Fr., *P.*, *C.*

## GASTROMYCETES.

- Mutinus caninus* (Huds.) Fr., *P.*  
*Phallus impudicus* (Linn.) Pers., *P.*, *K.*  
*Crucibulum vulgare* Tul., *K.*  
*Lycoperdon caelatum* (Bull.) Fr., *H.*, *perlatum* Pers., *P.*, *C.*, *K.*, *pyriforme* (Schaeff.) Pers., *E.*, *P.*, *C.*, *K.*  
*Scleroderma aurantium* Pers., *P.*, *C.*, *K.*, *Geaster* Fr., *P.*, *C.*, *K.*

## UREDINEAE.

- Uromyces Fabae* (Pers.) de Bary, on *Vicia sepium*, *P.*, *Valerianae* (Schum.) Fuck., *C.*, *Scrophulariae* (DC.) B. & Br., *E.*, *Poa* Rabenh.\* (*Aecidium* on *Ranunculus*), *P.*  
*Puccinia Violae* (Schum.) DC., *H.*, *C.*, *K.*, *Lychnidearum* Link\*, *P.*, *Malvacearum* Mont., *Dublin*, *Circaeae* Pers., *E.*, *P.*, *Saniculae* Grev., *E.*, *P.*, *K.*, *obtegens* (Link) Tul., *E.*, *Glechomatis* DC., *P.*, *Buxi* DC., *C.*, *K.*, *obscura* Schroet., *S.*, *Caricis* (Schum.) Rebent., *K.*, *holcina* Erikss., *H.*, *Poa* Niels. (*Aecidium* on *Tussilago*), *E.*, *H.*, *C.*  
*Triphragmium Ulmariae* (Schum.) Link., *C.*  
*Phragmidium Fragariastris* (DC.) Schroet. on *Potentilla reptans*, *K.*, *subcorticium* (Schränk) Wint., *P.*, *H.*, *C.*, *violaceum* (Schultz) Wint., *E.*, *P.*, *H.*, *Rubi-Idaei* (Pers.) Karst., *C.*  
*Coleosporium Petasitis* de Bary, *P.*, *C.*, *Tussilaginis* (Pers.) Kleb., *E.*, *P.*, *H.*, *C.*, *K.*  
*Pucciniastrum Circaeae* (Schum.) Schroet., *P.*, *C.*, *K.*  
*Milesina Dieteliana* Magn., *P.*, *Scolopendrii* (Fuck.) Jaap\*, *P.*, *H.*, *C.*  
*Melampsora Hypericorum* (DC.) Schroet., *K.*  
*Melampsoridium betulinum* (Pers.) Kleb., *C.*, *K.*  
*Melampsorella Caryophyllacearum* (DC.) Schroet.\* (= *Aecidium elatinum* A. & S.) on *Abies pectinata*, *K.*, *A. cephalonica* & *A. Lowiana*, *Avondale*.

## USTILAGINEAE.

- Ustilago Scabiosae* (Sow.) Wint., *K.*

## PYRENOMYCETES.

- Sphaerotheca pannosa (Wallr.) Lév., *K.*  
 Uncinula Aceris (DC.) Sacc., *P., H., C.,* & Longtown, Co. Kildare.  
 Microsphaeria Euonymi (DC.) Sacc., *C.* (perithecia), quercina (Schw.) Burr., *C., K.*  
 Erysiphe Cichoracearum DC. on *Taraxacum, H.,* Polygoni DC. on *Heracleum, E., H., K., Angelica, E., Circaea, P., Trifolium* spp., *H.,* and *Lotus corniculatus, K., graminis* DC., *E., C.*  
 Nectria coccinea (Pers.) Fr., *K., cinnabarina* (Tode) Fr., *H., C., K.*  
 Hypocrea rufa (Pers.) Fr., *P.*  
 Claviceps purpurea (Fr.) Tul., *C.*  
 Chaetomium elatum Kunze, *C.*  
 Stigmathea Robertiani Fr., *C., K.*  
 Mycosphaerella Fragariae (Tul.) Lindau\*, *P., Iridis* (Awd.) Schroet.\*, *K.*  
 Venturia Rumicis (Desm.) Wint., *P.*  
 Ophiobolus porphyrogonus (Tode) Sacc.\*, *C.*  
 Diaporthe taleola (Fr.) Sacc.\*, *C., Aucubae* Sacc.\*, *Avondale.*  
 Eutypa lata Tul., *C.*  
 Diatrype disciformis (Hoffm.) Fr., *H.*  
 Diatrypella quercina (Pers.) Nke., *C.*  
 Hypoxylon multifforme Fr., *C.*  
 Xylaria Hypoxylon (Linn.) Grev., *P., H., C., K.*  
 Ustilina vulgaris Tul., *E.*  
 Phyllachora graminis (Pers.) Fuck., *C., K.*  
 Endodothella Junci (Fr.) Theiss. & Syd.\*, *P.*

## HYSTERIACEAE.

- Rhophographus Pteridis (Sow.) Wint., *S., P., H.*

## DISCOMYCETES.

- Helvella crispa (Scop.) Fr., *P., C.*  
 Aleuria vesiculosa (Bull.) Boud., *H.*  
 Otidea onotica (Pers.) Fuck., *C., leporina* (Batsch) Fuck., *C.*  
 Peziza aurantia Pers., *P.*  
 Ciliaria scutellata (Linn.) Quél., *H., C.*  
 Coprobia granulata (Bull.) Boud., *C.*  
 Calycella citrina (Hedw.) Quél., *C.*  
 Coryne sarcoides (Jacq.) Tul., *P., C., K.*  
 Orbilia xanthostigma Fr., *H., C.*  
 Sclerotinia Curreyana (Berk.) Karst.\*, *P., H.*  
 Phialea firma (Pers.) Gill., *P.*  
 Chlorosplenium aeruginosum (Oeder) de Not. (mycelium only), *C.*  
 Helotium herbarum (Pers.) Fr., *C., fructigenum* (Bull.) Fuck., *H., C.*  
 Dasyscypha virginea (Batsch) Fuck., *P., C.*  
 Trichoscypha calycina (Schum.) Boud., *P.*  
 Mollisia cinerea (Batsch) Karst., *S., P., H.*  
 Phacidium multivalve (DC.) Kunze & Schm., *P.*  
 Pseudopeziza Trifolii (Biv.-Bern) Fuck., *H., K., Ranunculi* (Wallr.) Fuck. on *Anemone, C.*  
 Stegia Ilicis Fr., *E., P.*  
 Colpoma quercinum (Pers.) Wallr.\*, *C.*  
 Rhytisma acerinum (Pers.) Fr., *E., P., H., C., K.*

## PHYCOMYCETES.

- Syzygites megalocarpus Ehrb., *P., K.*  
 Cystopus cubicus (Strauss) de Bary (= *C. Tragopogonis* Schroet.), *K.*  
 Phytophthora infestans (Mont.) de Bary, *E., P., K.*  
 Plasmopara nivea (Ung.) Schroet. on *Heracleum, P., K.*  
 Bremia Lactucae Regel on Lettuce, *Dublin.*  
 Peronospora Potentillae de Bary\*, *P., K., alta* Fuck.\* on *Plantago media, C.*

### PROTOMYCETACEAE.

*Taphridium umbelliferarum* (Rostr.) Juel\*, on *Heracleum*, H.

### SPHAEROPSIDEAE.

- Phyllosticta hedericola* Dur. & Mont.\*, E., H., K.  
*Phoma samararum* Desm.\*, P., leucostigma (DC.) Sacc.\* on *Rhododendron*, C.  
*Rhizosphaera Kalkhoffii* Bubák\*, on *Picea pungens*, Glasnevin.  
*Ascochyta Nymphaeae* Pass.\*†, Glasnevin.  
*Darlucula Filum* (Biv.) Cast.\* on *Puccinia Caricis*, K.  
*Actinonema Rosae* (Lib.) Fr., Dublin, H., K.  
*Septoria Hederæ* Desm.\*, P., H., Rosae Desm.\*, K., Rubi West.\*, E., P.  
*Coniothyrium Fuckelii* Sacc.\*, H.  
*Gloeosporium Helicis* (Desm.) Oud.\*, H.  
*Marssonina Potentillae* (Desm.) Magnus\*, K.  
*Steganothecium pyriforme* (Hoffm.) Sacc.\*, Malahide.  
*Vermicularia Dematium* (Pers.) Fr., H., trichella Fr.\*, H.

### HYPHOMYCETES.

- Trichoderma viride* (Pers.) Fr., K.  
*Botrytis cinerea* Pers. (sclerotia on *Scilla nutans*), P.  
*Sepedonium chrysospermum* (Bull.) Fr., E.  
*Ovularia obliqua* (Cooke) Oud.\*, E., P., K., Glasnevin.  
*Ramularia Ajugae* (Niessl.) Sacc., P., calcea (Desm.) Ces., E., P., C., K.,  
*Cirsii* Allesch.\*, P., *Epilobii* Allesch.\*† on *Epilobium hirsutum*, C., *Heraclei*  
 (Oud.) Sacc.\*†, H., *Hypochoeridis* Magn.\*, K., *filaris* Fres. var. *Lappae*  
 Bres.\*, P., *lactea* (Desm.) Sacc.\*, P., K., *plantaginea* Sacc. & Berl.\* on  
*Plantago lanceolata*, K., *Primulae* Thuem.\*, E., P., C., K., *sambucina*  
 Sacc., P., *Scrophulariae* Fautr. & Roum.\*, E., *Urticae* Ces., E., *variabilis*  
 Fuck.\*, P., *nymphaearum* (Allesch.) Ramsb., on *Nuphar*, Glasnevin.  
*Cladosporium herbarum* (Pers.) Link, E.  
*Bispora monilioides* Corda, P., K.  
*Fusicladium depressum* (B. & Br.) Sacc., on *Angelica*, E.  
*Polythrincium Trifolii* Kunze, K.  
*Cercospora Opuli* (Fuck.) v. Hoehn., C.\*†  
*Fumago vagans* Pers., Dublin.  
*Tuberculina persicina* (Ditm.) Sacc. on *Uromyces Scrophulariae*, E.

## LICHENS OF THE DUBLIN FORAY.

By A. L. Smith and M. C. Knowles.

THE Dublin Foray was, as far as lichens are concerned, most wisely organised: the thanks of the lichenologists are due to the members of committee who selected districts that in their conditions of atmosphere and temperature were so well suited to lichen growth. The expeditions were planned so that the members were taken right out into the country away from the smoke of the city, and opportunity was given for collecting both rock and tree specimens: each day's excursion was marked by some special feature of open dale or of enclosed woodland with its particular lichen flora and in all the districts lichen growth was abundant and varied.

Before the main party arrived a visit was made by a few of the members to that part of the Dublin Mountains called the

Scalp and to the beautiful Glensink ending up at the village of Enniskerry. The boulders that were "confusedly hurled" over the hills at the Scalp were worked over, as far as was possible, in the limited time: they were covered with crustaceous forms in great white or dark masses, with many grey patches of leafy forms such as *Parmelia saxatilis*, the isidiose form of that species, there and elsewhere being unusually abundant. Some tree specimens were secured in Glensink and Rhizocarpons and Verrucarias from stones on the banks of the stream.

Powerscourt Demesne was the field of the first whole-day excursion. The ancient trees in the park and near the waterfall bore great sheets of *Lobaria pulmonaria*, *Cetraria glauca* and several of the larger *Parmeliae*, with other crustaceous, leafy or shrubby lichens on the trees, rocks or walls. *Pertusariae*, notably *P. pertusa* and *P. globulifera*, were abundant.

Carton Demesne was next visited: before leaving the highroad a bridge and a wall of limestone held the attention of the lichenologists a considerable time. White masses of *Aspicilia calcarea* which might have been aptly designated "spill o' the pail" alternated with the dark characteristic lime-loving Collemas and Leptogiums, the black *Placynthium nigrum* and brightly coloured orange species of *Placodium*. Within the Demesne and on the way up the lovely wooded glen many trees were examined and yielded species of *Graphideae* and Pyrenolichens with a few *Lecanorae* and *Lecideae*. And here may be pointed out the rare occurrence of *Lecanora varia* so abundant in the woods near London; *Lecidea parasema* was also rarely seen. At a further stage in the walk overhanging masses of lime cliffs by the side of the path were dotted with *Verrucaria* fructifications and adorned with several species of glowing orange *Placodii*. One small section was covered with crowded whitey-grey growths of *Dermatocarpon miniatum*, almost the colour of the cliffs when gathered, but changing to a darker brownish hue in the collecting boxes. The difficulty in many instances was to secure sufficient material of the minute forms for microscopic examination in the laboratory: hammer and chisel were almost unavailing against the smooth hard rock face.

The final excursion was to Kilruddery, where again we had most cordial and kind permission to go through the grounds. On the low wall of an ancient fountain pool were found *Physcia tribacia* and *Ph. orbicularis* with var. *virella* in closely appressed patches. The chief collecting there, however, was done on trees. Some old and massive trunks were found to be covered with a variety of crustaceous forms; but the outstanding species at Kilruddery was *Pyrenula nitida* which covered trunk after trunk of a long avenue of limes. Other crustaceous lichens such as

*Bacidia luteola*, *Lecanora rugosa*, *Pertusariae*, etc., were gathered, but *Pyrenula* was the most abundant of all. Kilruddery lies in the heart of the mountains and there as on the other excursions we were gladdened by views on every side of that most beautiful tract of country.

The arrangement followed in this list is that of *The Handbook of British Lichens*. The letters which come after the names of the species and varieties signify the localities in which the lichens were collected, viz. S. = Scalp, G. = Glensink and Killegar, P. = Powerscourt, C. = Carton and K. = Kilruddery. With the exception of Carton, the seat of the Duke of Leinster, which lies inside the Co. Kildare border, all these places are in Co. Wicklow.

Altogether 191 species and varieties have been identified, of these five are now recorded from Ireland for the first time, viz. *Biatorella flava*, *B. privigna*, *Biatorina Bouteillei*, *B. graniformis* and *Verrucaria parva*. Much of the success both of collection and determination of species is due to the untiring interest of Mr Paulson who was fortunately able to be with the party on two of the chief excursions.

#### EPHEBIACEAE.

- Placynthium nigrum* S. F. Gray, S.,  
P., K., C.  
*P. nigrum* S. F. Gray var. *psotina*  
Hue, K., C.

#### COLLEMACEAE.

- Collema pulposum* Ach., P., C.  
*C. multifidum* Schaer., C.  
*C. multipartitum* Mudd, C.  
*Leptogium sinuatum* Massal., P.  
*L. lacerum* S. F. Gray, C.  
*L. lacerum* var. *pulvinatum* Koerb., C.

#### PANNARIACEAE.

- Pannaria rubiginosa* Del. var. *conoplea* Koerb., P.

#### PELTIGERACEAE.

- Peltigera canina* Willd., S., G., P.,  
K., C.  
*P. polydactyla* Hoffm., P.  
*P. horizontalis* Hoffm., P.

#### STICTACEAE.

- Lobaria laciniata* Wain., P.  
*L. pulmonaria* Hoffm., P., K., C.

#### PARMELIACEAE.

- Parmelia physodes* Ach., P., K.  
*P. physodes* var. *tubulosa* Mudd, P.  
*P. perlata* Ach., G., P., K., C.  
*P. caperata* Ach., P., K.  
*P. subaurifera* Nyl., C.  
*P. saxatilis* Ach., S., P., K., C.  
*P. saxatilis* f. *furfuracea* Schaer., P.

- P. sulcata* Tayl., P.  
*P. dubia* Tayl., P., K.  
*P. conspersa* Ach., S., K.  
*P. Mougeottii* Schaer., S.  
*P. exasperata* Carroll, P.  
*P. fuliginosa* Nyl., S., P., K.  
*P. fuliginosa* var. *laetevirens* Nyl.,  
P., K.  
*Cetraria glauca* Ach., P.

#### USNACEAE.

- Evernia prunastri* Ach., G., P., K., C.  
*Ramalina calicaris* Fr., P., K.  
*R. fraxinea* Ach., P.  
*R. fastigiata* Ach., P., K., C.  
*R. farinacea* Ach., P., K.  
*Usnea florida* Web., P., K.  
*U. florida* var. *hirta* Ach., P.

#### PHYSICIACEAE.

- Xanthoria parietina* Th. Fr., G., S.,  
P., K., C.  
*X. lychnea* Th. Fr., S.  
*Placodium callopismum* Mer., C.  
*Pl. murorum* DC., S., P., C.  
*Pl. cirrochroum* Hepp, C.  
*Pl. citrinum* Anzi, S., P., C.  
*Pl. incrustans* A. L. Sm., S., C.  
*Pl. aurantiacum* Anzi, ?  
*Pl. pyraceum* Anzi, S.  
*Pl. ochraceum* Anzi, C.  
*Pl. vitellinum* A. L. Sm., S.  
*Pl. ferrugineum* Hepp var. *festivum*  
A. L. Sm., S.  
*Pl. rupestre* Branth. & Rostr., S., P.,  
C.

*Candelariella vitellina* Muell-Arg., S.  
*C. epixantha* A. L. Sm., S.  
*Physcia pulverulenta* Nyl., P.  
*Ph. stellaris* Nyl., P., K.  
*Ph. hispida* Tuckerm., G., P., K., C.  
*Ph. tribacia* Nyl., K.  
*Ph. orbicularis* Dalla Torre & Sarnth., P., K.  
*Ph. orbicularis* var. *virella* Dalla Torre & Sarnth., K.  
*Rinodina demissa* Arn., S.  
*R. sophodes* Th. Fr., P.

#### LECANORACEAE.

*Lecanora subfusca* Ach., G., P., K.  
*L. subfusca* var. *chlaronia* Ach., P., K.  
*L. subfusca* var. *allophana* Ach., G., P., C.  
*L. rugosa* Nyl., K.  
*L. campestris* B. de Lesd., S., C.  
*L. gangaleoides* Nyl., S.  
*L. atra* Ach., S., K.  
*L. Hageni* Ach., G., P.  
*L. crenulata* Nyl., S.  
*L. pallida* Schaer., K.  
*L. carpinea* Wain., P.  
*L. galactina* Ach., S., C.  
*L. urbana* Nyl., C.  
*L. varia* Ach., G.  
*L. farinaria* Borr., G., P.  
*L. symmictera* Nyl., G.  
*L. polytropa* Schaer., S., P.  
*L. tartarea* Ach., S., P.  
*L. parella* Ach., S., P., K.  
*L. calcarea* Sommerf., C.  
*L. calcarea* var. *contorta* Hepp, C.  
*L. calcarea* var. *Hoffmanni* Sommerf., C.  
*L. gibbosa* Nyl., S.  
*Acarospora smaragdula* Massal., S.  
*L. albariella* A. L. Sm., K.  
*Icmadophila ericetorum* A. Zahlbr., S.  
*Haematomma ventosum* Massal., S.  
*H. coccineum* Koerb., S.

#### PERTUSARIACEAE.

*Pertusaria velata* Nyl., P.  
*P. globulifera* Nyl., P.  
*P. faginea* Leight., P.  
*P. multipunctata* Nyl., P.  
*P. pertusa* Dalla Torre & Sarnth., S., P., K.  
*P. ceuthocarpa* Turn. & Borr., S.  
*P. leioplaca* Schaer., P., K.  
*P. Wulfenii* DC., P., K.

#### THELOTREMACEAE.

*Thelotrema lepadinum* Ach., P., K.  
*Diploschistes scruposus* Norm., S., P.

#### CLADONIACEAE.

*Baeomyces rufus* DC., P.  
*Cladonia pyxidata* Hoffm., P.  
*C. fimbriata* Fr. var. *simplex* Wain., P.  
*C. cervicornis* Schaer., P.  
*C. cornuta* Fr., P.  
*C. subsquamosa* Nyl., P.  
*C. parasitica* Hoffm., C.  
*C. coccifera* Willd., S., P.  
*C. macilenta* Hoffm., S.

#### LECIDEACEAE.

*Gyalecta exanthematica* Fr., C.  
*G. Flotovii* Koerb., K.  
*Lecidea mutabilis* Fée, P.  
*L. Metzleri* Th. Fr., C.  
*L. ochracea* Wedd., C.  
*L. dubia* Hook., G., C.  
*L. parasema* Ach., G., P., K., C.  
*L. goniophila* Schaer., P.  
*L. leucophaea* Nyl., S.  
*L. rivulosa* Ach., S., P.  
*L. albocoerulescens* Ach., P.  
*L. solediza* Nyl., S.  
*L. confluens* Ach., S.  
*L. contigua* Fr., P.  
*L. contigua* var. *platycarpa* Fr., P.  
*Biatorella flava* A. L. Sm., P.  
*B. privigna* A. L. Sm., S., C.  
*B. pruinosa* Mudd, C.  
*B. simplex* Br. & Rostr., C.  
*Biatorina Bouteillei* Arn., C.  
*B. lutea* Arn., K.  
*B. diluta* Th. Fr., C.  
*B. lenticularis* Koerb., P., K., C.  
*B. lenticularis* var. *erubescens* Koerb., C.  
*B. chalybeia* Mudd, S.  
*B. graniformis* A. L. Sm., K.  
*Bacidea luteola* Mudd, K.  
*B. phacodes* Koerb., K.  
*B. umbrina* Branth. & Rostr., S.  
*Buellia canescens* de Not., P., K., C.  
*B. myriocarpa* Mudd, G., P., K.  
*B. spuria* Koerb., S.  
*Leciographa parasitica* Massal., P. on  
*Pertusaria pertusa*.  
*Rhizocarpon geographicum* DC., S.  
*R. calcareum* Th. Fr., C.  
*R. petraeum* Massal., S., P.  
*R. albostratum* Th. Fr., S.  
*R. confervoides* DC., G., P.

#### LECANACTACEAE.

*Lecanactis abietina* Koerb., P.

#### ARTHONIACEAE.

*Arthonia gregaria* Koerb., C.  
*A. gregaria* var. *kermesina* A. L. Sm., C.  
*A. astroidea* Ach., P., K.  
*A. radiata* Ach., S., P.



**GRAPHIDACEAE.**

- Ophegrapha herpetica Ach., *P.*, *K.*, *C.*  
*O. atra* Pers., *G.*, *P.*, *K.*, *C.*  
*O. vulgata* Ach., *G.*, *P.*, *K.*, *C.*  
*O. varia* Pers., *K.*  
*Graphis elegans* Ach., *K.*  
*G. scripta* Ach., *P.*, *K.*, *C.*  
*G. scripta* var. *pulverulenta* Ach., *P.*  
*Phaeographis inusta* Muell.-Arg., *G.*,  
*K.*  
*P. dendritica* Muell.-Arg., *K.*  
*Graphina anguina* Muell.-Arg., *P.*, *K.*  
*G. inustula* A. L. Sm. (On holly), *K.*

**CHIODECTONACEAE.**

- Enterographa crassa Fée, *P.*, *K.*, *C.*

**DERMATOCARPACEAE.**

- Dermatocarpon miniatum Th. Fr., *C.*

**VERRUCARIACEAE.**

- Verrucaria aethiobola Wahlenb., *S.*  
*V. submersa* Schaer., *G.*  
*V. papillosa* Ach., *G.*  
*V. nigrescens* Pers., *G.*, *S.*, *P.*, *C.*  
*V. coerulea* DC., *C.*

- V. glaucina* Ach., *C.*  
*V. fuscella* Ach., *G.*  
*V. maculiformis* Kremph., *C.*  
*V. Dufourii* DC., *C.*  
*V. muralis* Ach., *S.*, *P.*  
*V. rupestris* Schrad., *P.*, *C.*  
*V. rupestris* var. *subalbicans* Mudd, *C.*  
*V. integra* Carroll, *G.*, *S.*, *C.*  
*V. parva* Deakin, *C.*  
*V. calciseda* DC., *C.*  
*Thelidium incavatum* Mudd, *C.*

**PYRENULACEAE.**

- Acrocordia gemmata Koerb., *K.*, *C.*  
*A. biformis* Oliv., *G.*, *C.*  
*A. epipolaea* A. L. Sm., *C.*  
*Arthopyrenia epidermidis* Mudd, *K.*  
*A. punctiformis* Arn., *K.*  
*A. cinereopruinosa* Koerb., *G.*, *C.*  
*A. fallax* Arn. (On cherry), *K.*, *C.*  
*Microthelia micula* Flot., *K.*  
*Porina carpinea* A. Zahlbr., *K.*  
*P. chlorotica* Wainio, *G.*, *P.*  
*P. olivacea* A. L. Sm., *K.*  
*Pyrenula nitida* Ach., *G.*, *K.*

**MYCETOZOA OF THE DUBLIN FORAY.**

By G. Lister.

THE weather during the first half of September was fine and dry, and the rain that fell just before and during the Foray was too recent to provide the best conditions for the appearance of Mycetozoa. Twenty-seven species were obtained, two of which were new records for Ireland, and three were new to the sub-province "L. 2" of Leinster, which includes counties Wicklow, Dublin and Kildare, Queen's County and King's County.

The first day's expedition to Powerscourt Demesne was wet. A large sawdust heap formed of coniferous wood yielded abundance of *Cribraria piriformis*, a species not before recorded for Ireland: besides the troops of mature red-brown sporangia, which exactly matched the colour of the sawdust, was much of the slate-grey or blackish plasmodium. On the same heap were numerous aethalia, mature and immature, of *Lycogala epidendrum*, the coral-red veins of plasmodium could be traced half an inch down among the sawdust. On fallen oak wood, amongst grass, *Arcyria pomiformis* was found, a new record for L. 2. The afternoon of September 24th, spent on Howth, yielded only *Didymium squamulosum* and *D. Clavus*, the latter being new to L. 2. The expedition to the Carton Demesne was more repaying to the hunters for Mycetozoa. In woodland among moist herbage were many old stumps and logs, where besides

*Badhamia panicea*, *Trichia decipiens*, *Hemitrichia clavata*, *Perichaena corticalis*, etc., a large growth of *Trichia contorta* var. *inconspicua* was found, a new record for L. 2. September 26th was also wet, but the excursion to Kilruddery was distinguished by the discovery of *Lachnobolus congestus*, a new record for Ireland.

In the following list *P.* = Powerscourt., *H.* = Howth, *C.* = Carton, and *K.* = Kilruddery.

*Ceratiomyxa fruticulosa* (Muell.) Macbr., *C.*  
*Badhamia panicea* (Fries) Rost., *C.*  
*Physarum nutans* Pers., *C.*  
*Fuligo septica* (L.) Gmel., *P.*  
*Didymium difforme* (Pers.) Duby., *K.* *D. Clavus* (Alb. & Schw.) Rost., *H.*  
*D. squamulosum* (Alb. & Schw.) Fries, *H.*, *C.*  
*Stemonitis fusca* Roth, *P.*, *C.* *S. hyperopta* Meylan, *P.* *S. splendens* Rost. var. *flaccida* Lister, *P.*  
*Comatricha nigra* (Pers.) Schroet., *C.*, *K.*  
*Cribraria piriformis* Schrad., *P.* *C. vulgaris* Schrad., *P.* *C. argillacea* Pers., *P.*  
*Tubifera ferruginosa* (Batsch) Gmel., *P.*, *K.*  
*Reticularia Lycoperdon* Bull., *P.*  
*Lycogala epidendrum* (L.) Fries, *P.*  
*Trichia affinis* de Bary, *K.* *T. scabra* Rost., *C.* *T. varia* Pers., *K.* *T. decipiens* (Pers.) Macbr., *C.* *T. contorta* (Ditm.) Rost. var. *inconspicua* Lister, *C.*  
*Hemitrichia clavata* (Pers.) Rost., *C.*  
*Arcyria pomiformis* (Leers) Rost., *P.* *A. denudata* (L.) Wettst., *P.* *A. incarnata* Pers., *P.*  
*Lachnobolus congestus* (Somm.) Lister, *K.*  
*Perichaena corticalis* (Batsch) Rost., *C.*

## NOTES ON IRISH MYCETOZOA.

By G. Lister.

IN 1912 a list of the Mycetozoa found in Ireland up to that date was given in Part 63 of the Clare Island Survey, where 64 species are recorded. Since then, search for Mycetozoa has been made in many parts of the country by experienced workers among whom Miss M. Rea, Mr and Mrs Stelfox and Mr W. F. Gunn have been most active. Papers dealing with the results of their observations have appeared from time to time in *The Irish Naturalist* and other journals\*, from the perusal of which it may be gathered that only seeing eyes and favourable seasons are wanted to prove that Ireland is as rich in Mycetozoa as her neighbours. The recent visit of the British Mycological Society to County Dublin, and the work carried on since by Mr and Mrs Stelfox have led to further additions to the Irish list, which now stands at ninety-eight. Of these perhaps the most interest-

\* M. D. Stelfox, "Myxomycetes from the Dingle Promontory," *Irish Naturalist*, xxiv, No. 2 (1915). M. D. Stelfox and M. W. Rea give a list of Myxomycetes from North Ireland, *Annual Report of Belfast Naturalists' Field Club*, Ser. II, vol. VII, No. 2 (1914-15). M. W. Rea and M. D. Stelfox "Some Records of Irish Mycetozoa," *Irish Naturalist*, xxvi, No. 4 (1917). W. F. Gunn, "Some Irish Mycetozoa," *Irish Naturalist*, xxviii, No. 4 (1919).

ing species is *Diderma lucidum*, reported before for Ireland, but not confirmed till it was found in some abundance on mossy rocks within the spray of the Dargle Waterfall, last October. This rare species with its bright orange-red sporangia on slender black stalks, has been only met with before, with certainty, from North Wales where it has been found repeatedly since its discovery there in 1860. Growing near it, Mr Stelfox also found *Diderma ochraceum* and *Lamproderma columbinum* var. *brevipes*, both new records for Ireland. Other Irish gatherings of special interest are *Physarum pulcherripes* and *P. brunneolum*, both obtained by Miss Rea in County Down; the former is known elsewhere only from the United States, *P. brunneolum* has a wider range, but, as far as hitherto noted, a very discontinuous one, having been found in Portugal, New South Wales, California and Chili.

The following species and varieties have been recorded from Ireland since the appearance of the Clare Island Survey:

- Badhamia foliicola* Lister, *B. macrocarpa* Rost., *B. nitens* Berk.  
*Physarum pulcherripes* Peck, *P. murinum* Lister, *P. galbeum* Wing., *P. viride* Pers. var. *incanum* Lister, *P. pusillum* Lister, *P. brunneolum* (Phill.) Mass.  
*P. crateriforme* Petch, *P. stramineipes* Lister, *P. didermoides* Rost., *P. virescens* Ditm. var. *nitens* Lister.  
*Fuligo septica* Gmel. var. *candida* R. E. Fries.  
*Craterium aureum* Rost.  
*Diderma floriforme* Pers., *D. lucidum* Berk. & Br., *D. ochraceum* G. F. Hoffm. (*D. globosum* Pers., which appears in an early list, has not been confirmed.)  
*Didymium diffforme* Link var. *comatum* Lister, *D. nigripes* Fr. var. *xanthopus*, Lister.  
*Stemonitis hyperopta* Meylan, *S. splendens* Rost. var. *Webberi* and *flaccida*, *S. confluens*, *S. herbatica* Peck, *S. flavogenita* Jahn.  
*Comatricha nigra* Schroet. var. *alta* Lister, *C. elegans* Lister.  
*Lamproderma columbinum* Rost. var. *brevipes* G. Lister, *L. arcyronema* Rost.  
*Brefeldia maxima* Rost.  
*Cribraria piriformis* Schrad., *C. tenella* Schrad.  
*Dictydium cancellatum* Macbr. var. *fuscum* Lister.  
*Licea minima* Fries.  
*Dictydiaethalium plumbeum* Rost.  
*Liceopsis lobata* Torrend.  
*Trichia favoginea* Pers. (confirmed), *T. scabra* Rost., *T. contorta* Rost. var. *inconspicua*, *T. Botrytis* Pers. var. *munda* Lister, *T. floriformis* G. Lister.  
*Hemitrichia Vesparium* Macbr. (*H. Serpula* Rost. unconfirmed).  
*Arcyria ferruginea* Sauter, *A. incarnata* Pers. var. *fulgens* Lister, *A. pomiformis* Rost.  
*Lachnobolus congestus* Lister.  
*Perichaena vermicularis* Rost.  
*Dianema corticatum* Lister.

**PRESIDENTIAL ADDRESS.**

By J. Ramsbottom, O.B.E., M.A., F.L.S.

**THE TAXONOMY OF FUNGI\*.**

At the outset of my remarks I would wish to express my satisfaction that my fellow members should have considered me worthy of occupying the Presidential Chair in the year that has seen the first Imperial Botanical Conference. We all hope that as a direct result of the conference a closer union in future will exist between botanists at home and those overseas and I trust that our Society will take an active part in all such Imperial efforts with a view to carrying out our chief aim—the study of mycology in all its branches. International relations are not yet all that can be desired but we as mycologists will lose nothing at any future International Congress if we can point to efforts made to further to its greatest extent the study of mycology within the Empire.

Shortly after our last Annual Meeting our Society suffered a great loss by the death of Sir Henry Hawley. An amateur in the truest sense of the word he was busily engaged for several years in preparing a monograph of the British Pyrenomycetes: it is much to be regretted that his manuscript was not left in a sufficiently advanced state to be completed for publication.

In considering matter for an address I was faced with the usual problem of choosing between discoursing on some general aspect of our studies and devoting myself to attempting to get clear in my own mind certain more or less floating ideas on the branch of mycology with which I am mainly occupied. The characteristic of our Society most commented on is the extreme friendliness of its proceedings and in making the choice of *The Taxonomy of Fungi* I trust I shall not be accused of attempting to spread discord in our ranks nor of taking undue advantage of brief authority to make statements which, by a thoughtful foresight on the part of those wise people who first arranged these matters, cannot be the subject for open discussion.

The taxonomist in mycology may at one time have been above suspicion but during the last few decades his position has been repeatedly assailed. One of the most amusing aspects of many discussions is that systematists are supposed either to be all in absolute agreement or to differ so radically that no botanist of average intelligence can be expected to pay the slightest attention to their deliberations. The fact of the matter is that

\* Delivered at Bettws-y-Coed, September 1924. The address was given as it stands except for a few verbal alterations and the addition of the footnotes.

there is just the same agreement and disagreement among systematists as among physiologists, cytologists, anatomists, or even, to come a little nearer home, phytopathologists.

As a systematist is traditionally an individual doomed to pass a secluded existence in a herbarium it may perhaps not be amiss first to consider such an institution.

The word herbarium apparently does not now hold the honoured position it did formerly. In the last half century its prestige was usurped by the laboratory which in turn seems to be giving way to that somewhat vague entity known as the "field." Much credit is due to the band of young botanists of the latter half of the nineteenth century who, fired with the teaching they had received in German laboratories, revolutionised botanical teaching in this country. It can truthfully be said, however, that their influence has not been wholly for good. The time was when systematic botany was so neglected in most of our Universities that it was not considered necessary to be able to recognise any plant other than the few mis-called "types"—and these rarely in a living condition or even whole. A student was encouraged to construct phylogenetic trees and was *au fait* with all the most recent theories of evolution and the origin of species, but the identification of species or the study of specific differences was usually totally ignored. The great value of the introduction of the study of ecology into the Universities has been that students have been driven to consider plants as they grow. It is to be hoped that the time is not far distant when a graduate on leaving a university will not feel that he cannot make original contributions to botanical science on account of not having an up-to-date laboratory at his disposal and spirit material of the rhizome of some tropical plant: there is much interesting work to be done *e.g.* in mycology, for which merely the outlines of methods and the necessary standard works need to be indicated. The day of the amateur, trained or not, is far from over.

A herbarium, or, as it was formerly called *Hortus siccus*, is a collection of dried plants; named plants arranged on some system. The use of such a collection in most groups is obvious and it may be said that the ordinary herbarium methods of drying and mounting are more or less satisfactory for fungi with the exception of fleshy agarics and some of the saprophytic filamentous forms. The drawback, of course, is that herbarium plants cannot be studied in a living condition. A herbarium is purely and simply a convenience. Certain facts of structure may be made out from properly dried specimens almost as well as from fresh material, but such specimens have their limitations.

Let us first consider the arrangement of the specimens in a

herbarium. In my opinion the chief desideratum about an arrangement is that it should be the most convenient one. If an alphabetical or a numerical arrangement proved to be the most satisfactory there would not be the slightest reason against adopting it. As a matter of fact most mycological herbaria are arranged by Saccardo's *Sylloge Fungorum*. This work is used because it contains (or is reputed to contain) all the species\* of fungi described up to 1910-11†. Many students taught on the Engler-Prantl System, or even on more modern lines, are surprised when a genus or species in which they are interested is not to be found in what is thought to be its natural position. Saccardo's classification is doubtless artificial and does not take into account the various rearrangements which research suggests would more nearly represent a natural order. Engler and Prantl's on the other hand was supposed to arrange the various genera according to their natural affinities and the second edition which is promised in three volumes, one of which is to appear in each of the next three years, will doubtless show many radical rearrangements. It is obvious, however, that a herbarium cannot repeatedly be disorganised in order to show continuous change in ideas—particularly when there is not always unanimity concerning the validity of the suggestions. The main point, however, is that no work other than Saccardo's attempts anything like a complete enumeration of species. The *Sylloge* has very many uses in a herbarium but the chief one is as an index. Mycologists are indeed in a fortunate position in having such a compilation for, on account of the enormous number of descriptions scattered in every type of publication, the life of a general systematist would otherwise be even less enviable than most people imagine it to be. Since the last volume (xxii) appeared the number of proposed new species seems to be increasing in geometrical progression and it is much to be desired that the promised continuation of this monumental work, which is really a complete mycological flora, will not be long delayed‡.

To understand the working of a herbarium it is necessary to realise that there are two classifications—a classification of convenience which enables one to find a given specimen‡ with the greatest ease, and a theoretical one which aims at arranging

\* This holds for all ranks. Consequently it is important that it should be realised more generally that a new genus obtains no mention in the *Sylloge* if it is based on one or more species whose descriptions have already been given. It is not sufficient, therefore, to rely on the generic indexes of this work when wishing to evade the perpetration of homonyms.

† A further volume of the *Sylloge* appeared in 1925, the first of three which are designed to include species described up to 1920.

‡ Or name a given specimen; cf. artificial keys.

plants according to their affinities. It may be pointed out that it was not until the time of Linnaeus that it was recognised that an arrangement of convenience was not necessarily the natural one.

In the same way as there are two kinds of classification there are two kinds of species\*—taxonomic and natural. It is much simpler to define the first than the second but as a rule the two are not kept sufficiently distinct for obvious reasons. Systematic biology postulates the existence of natural species. The attempt to describe these gives the theoretical species of the systematist†.

Let us consider the naming of a specimen. On examination it is seen to be, say, an *Ascobolus*. The usual floras are consulted and no description is found there into which the characters will "fit" as we say, *i.e.* no description can be found giving the details of our specimen. Saccardo's *Sylloge* is gone through with no success and similarly the various periodicals which have appeared since the last volume of the *Sylloge*. The fungus does not agree with the description of any known species of *Ascobolus*. It is a "new species." (Though it is not what I am aiming at I might add, parenthetically, that there is no glamour about a new species—a new species is merely an organism of which no description can be found.) Now, having such a fungus, the usual custom is to describe it, *i.e.* draw up a diagnosis of what one considers the essential characters‡.

All specimens that agree with the details of a properly drawn up diagnosis comprise a species from a taxonomic point of view. Further knowledge may lead to the description being emended: the taxonomic species thus changes. The ideal aimed at, of course, is so to define our taxonomic species that they agree absolutely with natural species, but for clarity it is essential that the two concepts should be kept distinct. It is the changing of the

\* I would express my indebtedness to my colleague Mr A. J. Wilmott for several of the ideas here put forward. It is not possible to say at this date which of them are definitely his, but I wish to put on record my appreciation of the benefits I have derived from discussions we have had on matters of nomenclature and classification during fifteen years as colleagues and friends in the Botanical Department of the British Museum.

† Mr C. Tate Regan in his Presidential Address to Section D—Zoology, at the British Association, Southampton (1925), gave a definition of a species which is interesting from this point of view. "A species is a community, or a number of related communities, whose distinctive morphological characters are, in the opinion of a competent systematist sufficiently definite to entitle it, or them, to a specific name." By a community is meant "a number of similar individuals that live together and breed together": an attempt to explain what a competent systematist is, within the meaning of the definition, would have been of interest.

‡ A concise diagnosis is much preferable to the wordy descriptions which cover pages of text, and usually succeed in leaving the reader as perplexed as the writer.

boundaries of taxonomic species which leads to much confusion of thought quite apart from the well-known "lumping" and "splitting."

My friend Mr C. G. Lloyd has for many years had a fling at what he calls "advertisements" but which most of us call authorities. Doubtless many systematists are misguided—or shall we say human—enough to like to see their name, or sufficient of it to ensure recognition, appended to a generic or specific name, and possibly also claim credit for what is colloquially called "making a new species." But the describing of new forms is not the work of a closed corporation indulging in back-scratching. The authority helps to precise the name. As everyone knows there are rules of priority and the general assumption is that a name can only mean one organism: if the same name is proposed later for a different species or genus it is a homonym and is invalid, *e.g.* if *Septoriopsis* Fragoso and Paul 1915, *Septoriopsis* Stevens and Dalbey 1917 and *Septoriopsis* von Hoehn. 1920 signify valid genera *Septoriopsis* must be used in the sense Fragoso and Paul used it and other names must be coined for the later genera. At present other names have not been proposed\* but in any event the addition of the authority precises the meaning. Systematic mycology does not remain stationary however. In the attempt to make taxonomic species agree more closely with natural ones diagnoses are frequently emended by addition or subtraction. Even systematists do not always refer to an original description but take their definition from some modern work. As an example, if one is naming British Pyrenomycetes one would probably use Winter's monograph in Rabenhorst's *Kryptogamen-Flora*. A description being found which sufficiently fits the characters of the fungus the name and authority there given would be used without consulting the original diagnosis. Now the original diagnosis may be one that would not include the given specimen—or, on the other hand, may include many modern species. In my opinion the name of the fungus should have attached to it some indication of the source of identification if the original diagnosis has not been consulted. Let us suppose a *Clavaria* is to be identified: in Cotton and Wakefield's monograph, Rea's *British Basidiomycetae* and Coker's *The Clavarias of the United States and Canada*, several names, *e.g.* *C. cristata*, have a different significance. Is it not reasonable to say which diagnosis is being followed? A further point is that an author himself may emend his original diagnosis. For instance we cannot assume that from 1814 to 1877 in years of continuous research the illustrious Fries did

\* Since this was written Petrak (*Ann. Mycol.* xxiii, 1925, p. 69) has proposed the name *Cercoseptoria* for *Septoriopsis* Stevens and Dalbey.



not alter his ideas regarding very many species and genera. For exactness therefore we should say which of his descriptions has been used.

Whatever a species may be we have something definite in a description but it must not be the description after it has been through the vicissitudes of a century's mangling. That this cannot be regarded as a matter of indifference to laboratory workers may be seen by a consideration of *Humaria rutilans* studied cytologically by Guilliermond and by Fraser. There is some doubt concerning what *Peziza rutilans* Fr. really is and the differences of opinion have led to a totally different interpretation in Rabenhorst's *Kryptogamen-Flora* and in Masee's *Fungus Flora*. Miss Fraser fortunately took the usual continental view of this species and so a controversy about the nuclear phenomena in the ascus was restricted to interpretation of facts and not adulterated with a discussion as to personal veracity.

If the statement be made that such and such an interpretation of a name is followed one factor causing discrepancy in results tends to be eliminated. The casual identification of an organism which has been investigated for several months is far more usual than many would imagine, and cannot be too strongly deprecated. Taxonomists are particularly interested in species which have been thoroughly investigated for reasons which I shall consider later. As a practical course I would suggest that when an authority is given this should mean that the original diagnosis has been followed and this may be precised by quoting volume, page and date. If a more recent diagnosis be used the emending authority should be added. Most workers rely mainly on a certain work and so long as it is known what this is we can ask for nothing more exact. A list of Agarics for example, becomes much more intelligible if a note is added saying that Rea's *British Basidiomycetae* is followed and any deviations from this indicated.

Having precised a diagnosis we have something certain—but we may not know what it means. However, in describing a new species an author is usually basing his description on a certain specimen or specimens, and may give a drawing. The fact that examples are known in which there is no agreement between the three complicates matters but the application of a name in that case is purely a question of nomenclature. Now nomenclature has probably a worse reputation than any other branch of a systematist's work; name changing, or as Lloyd more graphically expresses it, "name-juggling" is popularly supposed to be its object—"ici on lit les petites-affiches." The study of nomenclature has its fascinations—its chief requisite is a legal mind. Its aim should be to stabilise the names of plants so that when we use a name we have an idea of its significance.

What significance is to be given to a name from the point of view of nomenclature? Taxonomy abounds in uncertainties but there are a number of points where we are secure. One of these, if it exists, is the specimen or specimens used by an author in describing the species. For reason of greater certainty one specimen ought to be indicated by the proposer of the species as his type\*. Then whatever range is given to the name that particular specimen is included: the name stands or falls by that specimen. This application of the type method has been extended by many systematists to genera also; there is a type species in a genus and throughout any changes made in the limits of that genus, the type species must remain. It seems to follow logically that the type of a genus depends ultimately on the type specimen of the genus. Type† is not a satisfactory word—a type specimen does not mean a typical specimen but the original specimen or one chosen in its place. Some of the disadvantages resulting from the neglect of adopting such a type method have recently been given by Shear in studying the genus *Phoma*. The International Rules of Botanical Nomenclature read: "When a genus is divided into two or more genera the name must be kept and given to one of the principal divisions." By this method, as the heterogeneity of the genus is realised, a number of species are taken away to form a new genus and the older name is retained for the major group: this goes on in some of the larger genera until a residue of imperfectly known species is left to bear a well-known name although it may contain no species which the author of the genus himself included. This "method of remainders" or "residue method" leads to much confusion.

It is fashionable to affect a humorous attitude towards nomenclature but it is a remarkable fact that when overseas botanists were circularised to submit suitable subjects for discussion at the Imperial Botanical Conference more suggested nomenclature than the remaining botanical subjects put together. Consequently a small sub-committee was formed to consider whether changes in the International Rules were desirable.

\* The type concept is recognised in the International Rules of Botanical Nomenclature (1910). Article 39 Recommendation xviii *bis* reads: "When publishing names of new groups, one should indicate carefully the subdivision which one considers the nomenclatorial type of the group: the type genus of a family: the type species of a genus: the type variety or type specimen of a species. This precaution will avoid the nomenclatorial difficulties where, in the future, the group is to be divided." This is the translation given by Hitchcock in his *Descriptive Systematic Botany*, p. 159. Though he does not state that it differs from the "official" translation a comparison of the two reveals a difference in interpretation, which in this instance has been put into words.

† The name "standard" has been proposed by Sprague to replace type as it serves as a standard by which other specimens may be compared in case of doubt. It is not likely that this suggestion will be adopted as what is meant by type specimen and type method is well understood by systematists.

The following resolutions amongst others were adopted at the Conference:

1. Certain alterations should be made in the International Rules of Nomenclature.

2. Art. 36 (invalidating names of new genera published on and after January 1st, 1908, without Latin diagnoses) should be replaced by a strong *recommendation* to supply Latin diagnoses.

5. The principle of the type method of applying names should be formally accepted.

6. Art. 55, 2° (rejecting duplicating binomials, e.g. *Linaria Linaria*) should be revoked.

There were eight other resolutions and these will be brought forward as the agreed opinion of Empire Botanists at the next International Congress. Mr Sprague at our meeting in January of this year\* went into several of these matters. My reason for referring to them here is to draw attention to the special needs of mycology. New genera are being described by the thousand, new species by the hundreds of thousands and new strains threaten to reach their millions. He who pays the piper calls the tune. Mycologists are now turning the handle of the species mill to such purpose that we ought to have a voice in drawing up Rules. The next International Congress is to be held in Ithaca in June 1926†, and by that time mycologists ought to have well considered their position. Such matters as the type method are general but we have special difficulties in applying it‡.

Moreover are we satisfied with the different starting points for different groups? At Brussels this was carried by 130 votes to 4 but after fourteen years' experience are we agreed that it would not be better to begin all mycological nomenclature with Persoon or even Linnaeus? There are many matters arising from the prevalence of pleomorphy some of which will be mentioned in the course of my remarks. All such difficulties should be brought forward for proper International consideration; it is surprising how discussion frequently leads to agreement or at least compromise.

We have now two definite things—a description and a type specimen (for of course the type method implies that there *must* be a type in existence; if the author has not chosen one it is better for his followers to denote one than to be eternally worrying about it). Taxonomy, however, is not concerned only in dealing with type specimens. We all of us are agreed that there are such things as natural species. Though unhappy with

\* 1924.

† The date was afterwards altered to August. No decisions on nomenclature are, however, to be made.

‡ It will probably be necessary to modify the type method for certain groups of fungi.

*Cortinarius* each and every one of us would guarantee to spot *Amanita muscaria* and *Coprinus comatus* and have a good shot at such polymorphic forms as *Armillaria mellea* and *Laccaria laccata*.

The name, description and type specimen of a fungus are to be as exact as possible and the first two are to mean one thing and one alone. To identify various fungi, to group together and to alter descriptions until finally the taxonomic species really becomes that indefinable something we know and recognise in the field is one of the aims of taxonomy. And from a practical point of view a systematist endeavours to be able to give a name to any fungus in any condition. The other aim of taxonomy is to arrange genera and species into groups giving some idea of relation. Most systematists believe in evolution but though this hypothesis is the thread running through all modern schemes of classification taxonomic botany is not concerned with the how and wherefore. Systematists naturally have ideas on the subject of evolution seeing that they are constantly engaged in studying organisms but it does not seem to me that an attempt to solve the problem of the origin of species or of larger or smaller groups is the work of the taxonomist.

Every investigator in biology sooner or later hits up against matters of taxonomy. Frequently his first working acquaintance with the subject gives him rather a bad impression of it. It may be that he is interested in some special aspect of a problem. He may wish to identify a fungus which he has been investigating from some special point of view. He may realise that to say that an unnamed fungus does this, that or the other is not of very great use and therefore wishes to say definitely what the fungus is. As a result of his investigations he has found that his fungus takes on many forms, or produces some peculiar chemical compound, or has some strange property which, from his point of view, should enable one to name the fungus immediately. But the particular information he has acquired may be useless in affording one a clue to its identification: unless similar work has been done no hint can be given him\*. In such cases the species should be described as new.

I am of the opinion, despite the many moanings to be heard, that we are better situated in mycology than are taxonomists in any other group of plants. There is an absolute jumble amongst fungi at present, far more so than when Linnaeus placed them in his class CHAOS. My reason for the statement is that we are

\* A problem often encountered is that of naming a sterile mycelium in culture. Frequently this is a timber-destroying organism. A most useful piece of work would be the growing of all such destructive agents from the spores or sporophores and tabulating their cultural characters.

gaining so much information about fungi in general—morphology, cytology, pathogenicity, chemistry and cultural characters.

Though we are told that species when run to earth differ in chemical properties, or physico-chemical properties, or these with the addition of vital force, or entelechy, from a practical standpoint we are driven to define them, at least broadly, from their morphology.

Even the most rabid experimentalist describes the species he is studying from its morphological characters which is of itself significant. But are we to ignore characters other than morphological? Mycologists at least would be very unwise to do so. What we require is as much information as we can get, of any and every kind, concerning any given organism. In ordinary herbarium work it is not always possible to carry out certain lines of investigation which one feels might give a clue to affinities. If time were no object, or in other words, if sufficient systematists could be endowed to carry out research in pure taxonomy, no doubt something might be done in this direction but as the matter stands the systematist must cull information from where he can and there is no mycological writing but what may give a clue. One of the great hopes of the systematist is the phytopathologist. Perhaps he, of all others, has in the past had most to criticise in the present state of systematic mycology. The liason which exists in our society is, I am certain, one of the surest signs that both subjects will advance in this country. Probably more trained botanists are engaged on plant disease investigation than on any other branch of botany: one of the reasons for this is, of course, the fact that the subject is what is called economic, which may be interpreted as meaning that even a trained botanist must have some means of sustenance. When a disease is being investigated the object is, as in all disease, to find a means of prevention or of cure. Theoretically, the so-called host is the more important of the two or more organisms concerned—leaving the investigator out of account—but the parasite must be studied in all its aspects. From a purely systematic point of view all fungi are of equal importance but we are gradually obtaining information about parasitic fungi which will eventually change our whole ideas of classification. For some recondite reason systematists are assumed to lament this. At many of our meetings some new fact is presented which at first sight does not seem to fit into the scheme of things—but why should we deplore the addition to our knowledge of the chemistry or pathogenicity of a form? Much of it is good-natured banter but it cannot be stated too plainly that any investigator who adds in any way to our knowledge of any fungus is supplying facts which may be useful in taxonomy.

What is required is sufficient detail to be able to identify the species investigated—or give its name with certainty—both of these are pure taxonomy—and any additional information may give a clue to relationship.

Up to the present I have dealt only with species in the old sense so far as natural species have been mentioned. As a unit of description I have taken a taxonomic species. In several recent mycological papers the ideas given forth by Lotsy, in his *Evolution by means of hybridization*, have received approval. Lotsy writes: "He who ventures to write on the origin of species, ought to define what a species is, so ought he to do who describes species, no matter whether he considers his task finished when the description has been made, or whether he intends to make use of the described species to build up a more or less elaborate system. In other words: the systematist, as well as the evolutionist, ought to state clearly what he means by a species." This, which reads somewhat like an undergraduate's essay, is followed by three definitions. The first is that of a Linneon: "To replace the term species in the Linnean sense, and to designate a group of individuals which resemble one another more than they do any other individuals."

It is stated that "to establish a Linneon consequently requires careful morphological comparison only."

The term Jordanon is used "to replace the term species in the Jordanian sense, viz. mikrospecies, elementary species, etc. and to designate a group of externally alike individuals which all propagate their kind faithfully, under conditions excluding contamination by crossing with individuals belonging to other groups, as far as these external characters are concerned, with the only exception of noninheritable modifications of these characters, caused by the influences of the surroundings in the widest sense, to which these individuals or those composing the progeny may be exposed."

"To establish a Jordanon, morphological comparison alone consequently does not suffice; the transmittability of the characters by which the form was distinguished, must be proved by experimental breeding."

The term Species is used "to designate a group of individuals of identical constitution, unable to form more than one kind of gametes; all monogametic individuals of identical constitution consequently belong to one species."

"To establish a species, neither morphological comparison alone, nor experimental breeding by itself is sufficient, nor are the two combined; hybrid analysis is required in addition."

Such a definition of a species is not one that systematists will accept. The term has never previously been regarded as of such

a narrow connotation and if it is ever used in this sense it will need to have Lotsy's name added to it unless his advice is followed and we all define what we mean by species. A species is what Lotsy calls a Linneon but few, if any, would be satisfied in these days with such a parody of it as Lotsy's definition suggests.

The attempt to apply Lotsy's terminology to fungi has not been very happy. In this country Dr Brierley adopted what he called "perhaps the main features of this as they apply to fungi" and as he clearly stated many of the difficulties of systematic mycology received a certain amount of support. The Linneon and Jordanon are given in Lotsy's sense but when we reach the "species concept" we have a change which is not commented on, and leaves us at least no better placed than before. The name species is still used "to designate a group of individuals of identical constitution." If we could satisfy ourselves as to identical constitution, or even constitution, we should be a long way on our journey. I am uncertain whether Brierley's definition is intended to go beyond that sentence. The following sentences read: "To establish a species neither morphological comparison alone, nor comparison of the morphological facies of the organisms on standardised series of culture media is sufficient. Analysis of physiological reaction to standardised conditions is required in addition." This to my mind in no way helps us to gain a clear idea of any unit we are in search of, and seems no more practical for fungi than is Lotsy's idea of the necessity of hybridisation to establish a flowering plant species. We know there are groups below the old Linnean species but a consideration of these may be left for a few moments. What I wish to say now is that the statement frequently made that mycological systematists are satisfied with gross morphology shows a ludicrous lack of knowledge not only of taxonomy but also of mycology. We are in danger on the one hand of being carried away by the real species monger who litters up pages with new species—new in that he never troubles to ascertain what his predecessors, his contemporaries or even himself have done—and on the other by those who, having hardly ever succeeded in identifying a fungus, tell us in elegant periods covering an echoing emptiness that the old masters of botanical science if not frauds and mountebanks were at any rate fools.

So far as I am aware small attention has been paid to hybrids among fungi from a general point of view.

Apart from some original assertions from Sir John Hill—a person somewhat ingenious in all matters pertaining to the imagination—little or nothing was done until the epoch-making work of Blakeslee in 1904. It is true that Saccardo had pre-

viously recorded a supposed hybrid between *Nectria Desmazieri* and *Lisea Buxi*. The reputed parents were growing on a box twig and amongst them there were abundant perithecia having in their lower three-quarters the beautiful red tissue of the *Nectria*, while the rest, which contained the ostiole, showed the azure blue of the *Lisea*. Whatever the nature of the fusions such an occurrence deserves further study.

Blakeslee's work is sufficiently well known not to require detailed mention. He found that though in certain Mucoraceae, e.g. *Sporodinia*, a spore sown from a sporangium could give rise to zygospores, in others, such as *Rhizopus*, a single spore gave only sporangia. Two different strains were found in such forms, one of which was given the sign (+) and the other (-), and it was only when two strains of opposite signs (or sex) fused that zygospores were produced. Where the mycelia are similar the fungus is said to be homothallic, where a (+) and a (-) strain exist the fungus is heterothallic. Considerable work has been done on these lines in Mucoraceae in the last twenty years and at present certain controversies are in progress regarding the constancy of these strains. Blakeslee's original strains of *Rhizopus nigricans* have retained their characters through about two hundred and fifty generations but some investigators consider that a series of transitions exist between homothallic and heterothallic forms. Whatever the facts, there may be sufficient morphological differences between the (+) and (-) strains of the same fungus for them to have been described as separate species, whereas, on the other hand, in some species, there are considerable differences between the different races of a single sex. Saito and Naganishi have recorded a true hybrid zygospore between different species of *Mucor* but it seems uncertain that the forms they worked with were really different species. The possibility of the existence of such hybrids is, however, not to be lost sight of for it has been shown repeatedly that the opposite sexes of different species are capable of showing an imperfect sexual reaction when grown in contact. A race may react with the opposite sex of another species under temperature conditions which do not allow it to form zygospores with its normal mate in its own species. Thus *Cunninghamella*\* will readily give imperfect hybridisation reactions with species of *Mucor* at temperatures below 20° C. but will not itself form zygospores at so low a temperature; while some *Mucor* species are weak in reaction when contrasted *inter se*. The vigour of the reaction therefore has no necessary connection with the taxonomic relationships of the forms

\* *Cunninghamella*, it may be remembered is so unlike the ordinary Mucoraceae that it was originally described as a Hyphomycete.



involved. What seems to follow from this is that there is something fundamental common to all the strains of one "sex."

Burgeff produced hermaphrodite mycelia by mechanically mixing the protoplasts of the vegetative mycelia of plus and minus strains of *Phycomyces*. Hermaphrodite zygospores were occasionally produced and scanty sporangia which soon gave plus and minus strains. Such mixochimeras, as he termed them, would probably disconcert others than systematists if they were found to occur naturally.

That attempts at what one might term illegal crossing may have unlooked-for results is shown by some further work of Burgeff on *Chaetocladium* and *Parasitella*. These are both parasitic on species of *Mucor*. Both form gall-cells which in the second genus becomes a thick-walled storage organ somewhat resembling an azygospore. The gall-cell contains protoplasm from both host and parasite. While both plus and minus strains of *Parasitella* parasitise both plus and minus strains of most *Mucor* species it was found that the plus strain of *Parasitella* would attack only the minus strain of *Absidia glauca* and conversely. It is suggested on this and other grounds that the parasitism of *Chaetocladium* and *Parasitella* is a sexual process and has arisen as an attempt at hybridisation.

The knowledge of heterothallism has been extended very much during the last few years and is now known to occur in practically every fungus group—Phycomycetes (both Oomycetes and Zygomycetes), Ascomycetes (Pyrenomycetes, Discomycetes and probably in yeasts) and Basidiomycetes (Ustilagineae and Hymenomycetes). To consider the various aspects of the matter would take me too far from the subject of my address but Kniep's work on *Aleurodiscus polygonius* will suggest the complexity that faces us; it is surprising how frequently a clear-cut story becomes more and more puzzling as further research is accomplished; Kniep was able to study the behaviour of the four spores of a basidium in this species as they are shot off simultaneously. As was to be expected, assuming the four nuclear divisions in the basidium to be reduction divisions and that the so-called sex of the strain was dependent on its nuclei, two of the spores were of one sex, and two of the opposite sex. Only two strains were obtained from any basidium but when the mycelia from the whole fruit-body were analysed there were found to be four types or sexes. Vandendries is able to explain some complicated results in *Panaeolus separatus* on the assumption of four different sexes.

Brunswick's preliminary account of his work on *Coprinus* shows that the four spores of one basidium may give mycelia all of different sexes and that geographical races may occur.

A fact brought out by Kniep is that a single spore of *Schizophyllum commune* can furnish a fruit-body apparently indistinguishable from that formed by the union of two mycelia except that it does not possess clamp connections. This, at first sight, might appear to be of little interest to systematists, but are there not discrepancies in the accounts of the number of spores on the basidium, as *e.g.* in *Hygrophorus conicus*, where the yellow form has two spores and a uninucleate mycelium, which led Maire to propose the genus *Godfrinia* for its reception and other similar phenomena which may find their interpretation along these lines?

A point concerning heterothallism which has struck many mycologists is that we may have here some explanation of that bugbear to systematists, the Fungi Imperfecti. The assumption always has been that this group is a dumping ground for unattached conidial forms. Sometimes they have been defined as Fungi with incomplete life histories but a saner definition would be that they are Fungi concerning which our knowledge of their life cycles is incomplete. Very many of the Fungi Imperfecti have been joined up to their perfect stage in the past and suggestions of such relations are scattered throughout literature. It seems to me, however, that it is not unlikely that the reason many of them remain isolated is that they are heterothallic and few attempts have been made as yet to try the effect of different strains on one another. I am not suggesting that success will attend every effort but I do think that in such fungi as *Sepedonium chrysospermum*, *Bispora monilioides*, *Coryne sarcoides* and others—even *Penicillium*\*, where the conidial stage is remarkably common and the reputed perfect stage sometimes very rare—we might look for heterothallism. Whenever culture work on so-called pure lines or similar investigations are being carried out I would suggest that the strains should be tested against each other and the result stated, if negative; presumably we should have no cause for anxiety about a positive result being disregarded. It is quite conceivable that evolution has proceeded in conidial forms. Is it possible that in some instances it has resulted in a loss of sex? If the Fungi Imperfecti contain unrelatable conidial stages that is presumably what has happened, for in our present state of knowledge we must assume that they were homothallic or heterothallic originally. Such facts that *Cunninghamella echinata* forms abundant sporangia above and below 25° C., but only forms zygospores above that temperature, indicates some of the difficulties. The Fungi Imperfecti have always annoyed systematists and, moreover, being the cause of most fungus

\* Dr Derx has proved *Penicillium luteum* to be heterothallic (see p. 107).

diseases, whether of plant or animal, and often being easy to culture have caused annoyance and despair in other circles. Academic mycologists as a rule utterly ignore them, a reasonable but not satisfactory procedure. The assumption in systematic mycology is that they form an artificial group and, as they do not possess a perfect stage, they have usually been classified in an appallingly artificial manner. Recently Vuillemin, von Hoehnel and others have attempted to instil a little more reason into their classification but at present the general belief is that a higher form may have conidial stages which are now placed in different conidial genera, and conversely that a given form-genus may be the conidial stage of more than one perfect form. With further knowledge of the relation between sexual and asexual stages the species of Fungi Imperfecti ought to be taken out of the group lock, stock and barrel and joined on to the perfect stage. It is only in this way that we can get rid of the idea that the Fungi Imperfecti form a group equivalent in rank to the other great groups\*.

As I mentioned above Fungi Imperfecti have been much studied in laboratories for various reasons. It has long been known, indeed since the earliest times of culture work, that fungi grown on media are extremely liable to take on forms not met with under natural conditions. As a consequence the results of culture work did not appeal to those systematists who conducted it. Further investigations, such as those of Matruchot thirty years ago, showed that when certain media were used a fungus might have its facies so altered that by a systematist it would be placed in another genus. It is now one of the best known features of culture work that the form of a fungus can be altered in all sorts of ways by the use of different media. Much has been made of this from a theoretical point of view. A popular method of representing a species nowadays is to write a little equation where  $a$  = the genotype,  $b$  the environment and  $ab$  the phenotype. This is a very deceptive method of representation as it simulates mathematical exactness. Granting for the moment that " $a$ ," the genotype, is a constant—a peculiar assumption concerning a living and consequently constantly changing organism, what of  $b$  the environment? Every one is agreed that the environment of a plant growing

\* As this stands it requires amplification. A natural classification would take note of conidial forms and treat them in connection with the perfect stage. A classification of convenience places conidial forms in form-genera in the Fungi Imperfecti. We might combine the two methods in our systematic works by uniting all stages of a fungus in one description and leaving the name of the imperfect stage or stages in the Fungi Imperfecti with cross references. It is not unlikely that with greater attention to the classification of the different sections of Fungi Imperfecti we shall gain much information concerning relationship.

under ordinary conditions would hardly be considered as a thing we know with any exactness. With a culture medium it is tacitly assumed that we have rigidly standardised conditions and might therefore with some hope of success begin to guess at that will o' the wisp "*a*." It is obviously possible to grow certain fungi in conditions that they are never likely to encounter in nature. Being able to bring about various alterations in the medium we are able to influence the growth of the fungus often in startling ways. But is ever such a medium constant? Potato, prune, and other natural substances are frequently used for media but it is beginning to be recognised that a synthetic medium is more likely to have a definite composition. We may know the composition of the agar we use and the exact percentages of the various chemicals before the medium is sterilised, we may assume that sterilisation always brings about the same changes, but does the medium remain constant when a fungus has begun to grow upon it? Often the change in the medium is visible to the naked eye and we know that staling products are poured out. A freshly made up medium differs often to a great extent from one which is a week old, and the humidity in a tube or Petri dish varies in amount. The only factors that can be regulated with certainty are probably temperature and light intensity.

A comparison of the growths of the two strains of a heterothallic species with what occurs on the same medium when both are present, and the comparison of the growth under the same conditions of a starved mycelium and one which has had a happier past would convince most mycologists that the mathematics of these things is more of the Einstein type than of the Hall and Stevens\*. What is always with us however is the *ab* and in the present state of knowledge we can hope for little more than to define this morphologically. Is it too much to ask that when an investigator is working with a fungus that can be cultivated he should, where possible, run his fungus through a few of the more common media and give data of the results? Almost every worker either modifies someone else's medium or makes up a fresh one—media-mongering is becoming as prevalent as species-mongering—and with the very best intentions it is not always possible to make up extravagant media; and so one does what one has to do with diagnoses in Russian and Japanese. In bacteriology, which, owing to the size of the organisms with which it is concerned, cannot gain much from morphology but has to define its species to a great extent from cultural characters, the Society of American Bacteriologists

\* Perhaps it should be explained that the Hall and Stevens mentioned are the authors of well-known English text-books of elementary mathematics.

has drawn up a descriptive chart after the method of the Dewey decimal system. No such method has yet been advocated for fungi. Lutz and Guéguen, however, read a paper at the International Congress of Botanists at Paris, 1900, suggesting that some scheme similar to one just previously proposed by Grimbert for bacteria should be applied to Mucedineae and yeasts. They proposed Raulin's solution as a standard medium and gave details of the preparation of fifteen modifications of this solution—also milk, potato, carrot and egg albumen. They even entered so far into details as to suggest the use of Erlenmeyer flasks, but the scheme appears to have appealed to none.

In 1908 Mangin investigated species of *Aspergillus* in culture. Struck with the state of affairs in that genus he suggested that the matter should be discussed by the mycologists at the Brussels Congress though I cannot find that this was done. His suggestion was that the diagnoses of species which could be grown in culture should be established as far as possible on the characters at the optimum of growth, they should be accompanied by temperature data and an indication of the amplitudes of variation according to the conditions of the medium. I should like to suggest that at the next International Botanical Congress a very appropriate subject for discussion would be that of the best methods of precisising diagnoses drawn up from cultures.

Another matter that might also be discussed with advantage is the nomenclature of ranks lower than variety. The special forms or biological races in Rust Fungi were considered to some extent at the last International Botanical Congress but matters have become rather more complicated since. The older view is well represented by Fischer, who accepted as species: (1) all forms that are structurally distinct; (2) all forms which have a different life cycle; (3) all forms which differ in their host plants in as far as the hosts belong to different genera; in heteroecious rusts species are recognised if one generation, aecidia, uredo or teleuto occurs on different genera of hosts.

The work accomplished, during the last year or two, principally in America, has shown that such a comparatively simple method of nomenclature is insufficient for practical purposes—and the essentials of nomenclature are that it should be practical and practicable. As an example of the present state of our knowledge *Puccinia graminis* var. *Tritici* may be taken. This consists of at least thirty-seven biological forms which can be distinguished readily from each other by their action on certain pure-line varieties of various strains of *Triticum*. Stakman states that "the longer one works with these forms, the deeper becomes the conviction that they represent as real, as constant, and as genetically pure entities as do morphological species."

The work of Klebahn, Stakman and others has shown, however, that such physiological species frequently exhibit morphological differences. The forms of *Puccinia graminis*, which are separable on the basis of their action on different genera of host plants, can be recognised by the size, shape and colour of the uredospores and also by the size of the teleutospores and aecidiospores provided these spores are developed on hosts of the same approximate degree of susceptibility, and under identical environmental conditions.

Work on Peronosporaceae has yielded similar results: Gäumann has recognised more than fifty such forms of *Peronospora parasitica* occurring on members of the Cruciferae. It may well be that such forms exist in many, if not all, parasitic groups. Their definition depends mainly upon the susceptibility of the different strains of host plant; it being assumed, on the evidence so far accumulated, that this susceptibility is constant under definite environmental conditions. The host therefore acts as a selective medium and we can compare this with Dox's results in *Penicillium* and *Aspergillus*, where it was found that some species can be recognised more easily by their effects on certain media than by morphological characters.

Single-spore cultures have shown that many species consist of a number of strains or pure lines. Such strains have long been known in yeasts, principally through the work of Will, but within recent years the work of Johannsen on pure lines has attracted workers in every branch of biology, and fungi, fortunately, have not escaped. The saprophytes capable of culture are attracting attention and as the experiments are easy to carry out we may hope for a rapid accumulation of data from post-graduate students. One of the conclusions of La Rue and Bartlett shows what we may expect. "It appears that by using a sufficiently refined technique a nominal species such as *Pestalozzia Guepini* might be resolved into an indefinite number of demonstrably distinct strains, the number depending only upon the precision of the methods." A further statement by these authors reads: "The species concept in the Fungi Imperfecti is of necessity a highly artificial one. The ultimate unit, an impracticable one for purposes of classification, is the pure line descended from a single spore." The treatment of such pure lines from a taxonomic standpoint depends upon whether they are indefinite in number or not: if they are indefinite we may ignore them, as for all practical purposes of classification they would be the same as individuals. (Incidentally one might ask whether we are quite sure what constitutes an individual in many fungi.)

What are loosely termed "mutations" are recorded by many

of the older writers. However, practically all workers on pure lines report permanent changes which occur either infrequently, as in *Pestalozzia* and *Mucor*, or commonly, as Stevens has recorded in *Helminthosporium* and Brown and Horne in *Fusarium*. It has been held by some that because no nuclear change is known to occur in the production of these forms they cannot be spoken of as mutants. Whatever we call them they occur and are frequently permanent\*. We know little or nothing more about them. Do they occur in nature or is growing a fungus in a culture medium to be compared with growing a wild plant in a flower bed? Personally I can gain no mental satisfaction in regarding protoplasm for one purpose as an extremely stable chemical compound differing in different species in some small constituent but unchangeable through the ages no matter to what conditions of life the organism is subjected, and for some other purpose regarding it as a very welter of colloidal chemistry. If there are differences in the mode of origin of saltations these may be useful in enabling us to distinguish different types and to assign to them different ranks.

What is to be done with these various categories? We have the ordinary systematic species at one time defined on purely morphological grounds for the sole reason that no other method was then possible. These must still remain the unit of classification—and however the physico-chemical advocates bemoan the fact they will still have to record their results in *Pestalozzia Guepini*, for example, or in *Sterigmatocystis niger*. Such species should, however, be precised in every possible way—whether the precision comes from chemical, physiological, anatomical, cytological, cultural or any other characters. Lumping and splitting are purely temperamental, but splitters will group their elementary species in some such way as Costantin and Lucat did the closely related species of *Aspergillus* of the *fumigatus* group for which they used Chauvaud's term *stirpe*.

Below the rank of species various grades are listed in the International Rules, subspecies, variety, subvariety and what not, which may, or may not, have their uses in mycology: most of them seem redundant and to depend for their definition on personal idiosyncrasy. Biological species should be given definite names, the rank depending upon whether both morphological and physiological characters are present or physiological ones alone. In this way we should be able to speak of them, but for clarity the systematic species into which they fall should be indicated. Pure lines with definite morphological or physiological characters require to be considered fully when we have

\* Their permanence is sometimes only relative depending upon the cultural methods adopted as e.g. Chaudhuri found in *Colletotrichum biologicum*.

more information as to their prevalence and importance from a practical point of view. It may be that, like cultivated flowering plants, they require names. Whatever we do I hope that such nomenclature as "*Torula anomala* x's strain No. i" will not be considered legitimate, although it is in essence the system adopted in Horticulture with its Madame Butterfly's and Potato Queens.

For saltants we should have a separate rank, regarding them as forms of the species from which they are found to have arisen and noting whether they are known in culture only. Their prevalence and permanency must decide their taxonomic treatment.

Taxonomy is—even over the ground to which I have restricted myself—a controversial subject, but taxonomists, even when they happen to be mycologists, claim that they are botanists. Botanists in these matters have a reputation which they must not besmirch.

Alphonse de Candolle, in his classical work *La Phytographie*, writes: "All science, including botany, elevates the character in that it requires an ardent love of truth and rests on the idea that authors are thoroughly truthful. Science in this world plays the part of a practical school of good faith. Following these reflections one can regard botanists as being ordinarily and of necessity always men of peace, inoffensive, indulgent of the mistakes of their colleagues, and occupied more with the advancement of science than with their own interests or petty distinctions."

So speaks a botanist, but he is not alone in his opinions. R. L. Stevenson tells us that "men who fish, botanise, work with the turning-lathe, or gather seaweeds, will make admirable husbands; and a little amateur painting in water-colour shows the innocent and quiet mind."

So liberally endowed by providence, it ought not to be beyond our powers of restraint to work in such unity that we may bring nearer to accomplishment the ideal of Asa Gray—"Botanical classification, when complete, and correct, will be an epitome of our knowledge of plants."

## LICHEN DYES.

By A. Lorrain Smith.

LICHENS are peculiar plants in that they are composite organisms formed of fungal hyphae and green or blue-green algae that live in symbiotic relationship, each partner or symbiont contributing to the other and to the general good of the whole plant the material necessary for life and development.



The dual life has been curiously successful: lichens are all thalloid plants and no great development on morphological lines has been reached, but structural adaptations of various kinds have been formed to meet the necessities of their growth. They have spread everywhere over the earth's surface: they grow freely in the tropics; they reach almost to the poles, where they flourish on any bare rock, and they have reached higher up the mountains than any other vegetation. This power of meeting all conditions is directly due to their composite nature; but another of their most striking peculiarities is the formation within their tissues of organic substances—lichen-acids—some of which yield brilliant colours and are used to dye the animal fibres silk and wool. They have also been employed in colouring marble and rubber goods and for staining bacteria.

Acid substances occur in lichens that are associated with bright green algae and usually in well-aerated and well-lighted parts of the plant. They are not produced by lichens in which the algal symbiont is blue-green (Collemaceae, etc.), though a substance peltigerin has been extracted from *Peltigera canina*, a blue-green species. Much work has been done by chemists on lichen acids and in 1907 Zopf published the results up to that date—a record of about 150 different kinds of which the properties and chemical formulae had been determined. The work still proceeds: Lettau, for instance, in 1914, recorded the presence of salazinic acid in 70 different lichens.

These peculiar substances, so far as known, are only found in lichens. An almost similar body occurring in rhubarb root, called chrysophanic acid was supposed to be identical with parietin, the yellow colour-substance of *Xanthoria parietina* and allied species, but the chemical formula is different.

The use of these lichen-acids as a dyeing industry dates to a remote age. It is supposed that Ezekiel referred to garments dyed with lichens when he denounced the luxury of Tyre: "blue and purple from the isles of Elishah was that which covered thee." Theophrastos and Pliny almost certainly indicated a lichen in the Phycos Thalassion, "with crisp leaves used in Crete for dyeing garments." The Phycos Thalassion of the Greeks is undoubtedly the lichen *Roccella* which grows on rocks by the sea. Its use as a dye substance came from the East to Greece and to Italy; afterwards the art seems to have been lost, then rediscovered in the fourteenth century by a Florentine merchant, Roderigo, who made a large fortune and founded the family of the Orcellarii or Rucellai, hence possibly the name *Roccella*, and the derivation of orchil.

The beautiful blue orchil dye is the most renowned and the most prized of all the lichen dyes, and in different countries has

been known as orseille (France), litmus (Holland), and cudbear (Scotland). The lichens from which it is extracted vary extremely in type but are all included under the term orchil "lichens." There are several orchil acids: erythrin ( $C_{20}H_{22}O_{10}$ ) from species of *Rocella*, lecanoric acid (orseillic acid ( $C_{16}H_{14}O_7$ )) from *Lecanora tartarea*, gyrophoric acid ( $C_{16}H_{14}O_7$ ) from *Umbilicaria pustulata* and evernic and ramalic acids (each  $C_{17}H_{16}O_7$ ), from *Evernia prunastri* and *Ramalina pollinaria*. By means of ammonia fermentation—a bacterial process—the natural acids are split into orcin and carbonic acid. Orcin in the presence of air becomes orceine, which is the principal ingredient of orchil. Zopf, in the work referred to, has divided lichen acids into two great organic series: (1) the Fat Series, (2) the Benzole or Aromatic Series. The latter is again divided into subseries: (1) Orcin-derivatives, and (2) Anthracene-derivatives. It is from orcin-derivatives that the blue dye-stuffs are obtained. Parietin belongs to the anthracene group. Zopf and others hold that the alga in its metabolism forms an alcohol, the fungal hypha provides the acid: from these two substances there is formed, as in lecanoric acid, an erythritester or erythrin. The lichen acids occur as an excretion and always on the outside of the hyphae. Though not always easy to see under the microscope, yet in some species, as in *Coniocybe furfuracea*, they form crystals in the lichen up to  $20\mu$  in length. Barbatin, also a colourless acid contained in *Usnea* spp., is also recognisable under high magnification, while several of the naturally coloured acids, such as parietin, are quite distinct. The names given to the different acids generally reflect either the generic or specific name of the lichen from which they were first obtained. Thus salazinic acid, now found in many of our common lichens, was first extracted from *Stereocaulon salazinum*, a rare African species.

*Rocella tinctoria* is the orchil species most used in southern countries; it was and is now obtained on the rocks bordering the Mediterranean; the best material is got from the Canary Isles, but it is collected from all the African coasts as well as from India and S. America. *Rocella tinctoria* is not a British lichen. Two species, *R. phycopsis* and *R. fucoides* grow sparingly on the rocky cliffs of S.W. England and they also yield erythrin but in less abundance.

Cudbear and litmus are chiefly manufactured from the crustaceous lichen *Lecanora tartarea* which yields lecanoric acid. It is one of the most wide-spread lichens and of fairly rapid growth, so that it soon covers large spaces of tree trunk, rock or soil with its light grey crust. It is a northern plant and is abundant in West Scotland and in the Scandinavian countries. From Norway and Sweden it was brought in ship-loads to

Holland for the preparation of litmus. Cudbear, the Scottish name, is a corruption of Cuthbert, Dr Cuthbert Gordon having started an industry in Glasgow to make use of the West Highland product. Lindsay, writing in 1868, said that orchil was unknown in Stornoway, but cudbear was kept on sale by every grocer and was used by the villagers to dye blankets, shawls, etc.

In Fair Isle, one of the Shetland group, Lindsay found that the colours used in dyeing, many of them from lichens, were bright and gaudy and that the patterns were peculiar to the island. Tradition points to the sailors from the wrecks of the Spanish Armada as the source of the teaching of these patterns, and it has been noted that the same type of colouring and of pattern is characteristic of the long stockings worn by the Spanish peasantry.

In the preparation of litmus or cudbear the orchil lichens are powdered and soaked in ammoniacal liquor for ten or twelve days; alum, potash and lime are added and the mixture is allowed to stand till the maximum degree of colour is observed. During this process—which is a bacterial fermentation—the mass should be constantly stirred to allow contact with the air. Other methods of making cudbear slightly varying in detail from the above are also recommended by different writers.

Litmus paper, which is tinted violet, is prepared by steeping specially manufactured paper in an aqueous solution of litmus. The name litmus or lakmoes is of Dutch origin from *lac*, a resinous dye substance, and *moes*, pulp.

Orchil colours are the most striking, but other beautiful and serviceable dyes have been prepared from a varied series of lichens. From yellow lichens, such as *Letharia vulpina*, a yellow colour is obtained and has been used to dye church candles. *Xanthoria parietina* with alum yields a yellow dye.

Yellow-brown is the colour given by salazinic acid (one of the fat series): it is the acid constituent of *Parmelia conspersa* and of many of our native lichens—though in most of them in too small quantities to be of much service.

Reddish-brown is obtained directly from the very common tree or rock lichen, *Parmelia saxatilis*; it is only necessary to fill a pot with alternate layers of lichen and the material to be dyed, cover with water and boil for some hours: the result is a beautiful and durable red-brown colour. *Lobaria pulmonaria*, *Usnea* sp. and other lichens also give various shades of brown according to treatment. A great deal depends on the quantity of lichens used, the time given to the ammonia fermentation and to the mordants employed. *Lecanora tartarea*, for instance, a true orchil lichen, in a successful experiment gave a delicate brown colour with a pinkish tinge after it had been fermented

in ammonia for ten days and then boiled with alum. It is possible to vary the tints also by an admixture of several kinds of lichen in the dye-pot and by boiling for longer or shorter periods.

As already stated dyeing with lichens was an ancient art, widely known and practised. In all works that touch on lichens from the herbals onward, there are constant notes referring to the dyeing properties of the plants described: thus Lightfoot (1777), in a casual note, comments on the use of *Parmelia omphalodes* from which the natives of the West Highlands obtained a brown dye. On the Continent the more important works directly dealing with dye-lichens are those of Hoffmann (1787) and Westring (1792) who published treatises on the subject illustrated by coloured rectangular blocks of the colours obtained. Slightly later, Westring (1805-9), a Swede, was wishful that his countrymen, in the absence of *Rocella*, should utilise the lichens they had in abundance to obtain the orchil colours: *Lecanora tartarea*, *Umbilicaria pustulata* and species of *Gyrophora* all grow freely in northern countries and all give the desired blue colour.

W. Lauder Lindsay was the first in our country to give serious attention to lichen dyes. He was a doctor in Perth who devoted much time to botanical studies, especially to the study of lichens. In 1854\* and 1855 he published an account of his *Experiments on the Dyeing Properties of Lichens*, and in 1856 *A Popular History of British Lichens*, with numerous coloured plates which proved for many of us a fascinating introduction to the study of lichens. Though interested in the group chiefly from a scientific point of view, he realised the importance of an organised collection of lichens both to the dyeing industry and to the poor inhabitants of the somewhat desolate regions of the West Highlands. In order to further the use of lichens he published practical instructions as to the kinds of lichen most suitable for purposes of dyeing:

1. That crustaceous dwarf pale-coloured species growing on rocks, and especially on sea-coasts are most likely to yield red and purple for the preparation of orchil, cudbear or litmus.

2. That the colour of the thallus is no indication of colorific power in orchil lichens, inasmuch as the colour substances are the results of chemical action (ammonia fermentation).

3. That alterations in physical characters, chemical composition and consequently in dyeing properties are very liable to be produced by modification in the following external circumstances: (1) degree of moisture; (2) degree of heat; (3) degree of exposure to light and air; (4) climate; (5) elevation above the

\* Edin. New Phil. Journ. LVII, pp. 228-249 and LVIII, pp. 56-80 (1855).

sea; (6) habitat (nature of basis or support); (7) age; (8) seasons and atmospheric vicissitudes.

August has been recommended as the best month for collecting; *i.e.* just after the season of greatest light and heat when the accumulation of acids will be at its maximum.

Considerable use has been made of the presence of acids in lichens as a method of determining species. The nature of the acid as evidenced by its colour reaction to alkalies or to calcium hypochlorite, and its occurrence either in the surface tissues or in the medulla have been brought into service by field workers. Nylander was the first to realise the value of the colour tests to systematists, and published a paper on the subject in 1866. It is only necessary in certain doubtful cases of determination to apply a drop of alkaline or calcium hypochlorite solution to secure decisive confirmation. The most efficient method is to place a minute portion of the plant in a water solution under the microscope; a drop of the reagent is introduced and the result however faint or elusive can be easily seen. Generally, however, it is sufficient to touch the plant with a rod dipped in the reagent. A number of species have, somewhat unfortunately, been based on the presence or absence of certain acids in the tissues, though, as a rule, morphological characters are present also.

## STUDIES IN ENTOMOGENOUS FUNGI.

(With Plate I and 1 Text-fig.)

### IX. AEGERITA.

By T. Petch, B.A., B.Sc.

IN 1896, H. J. Webber, while investigating the sooty mould of the orange in Florida, found that the larvae and pupae of the mealy wing (*Aleyrodes citri*) were attacked by a brown fungus, as well as by *Aschersonia*. The fungus formed a seal-brown pustule over the insect and ultimately entirely concealed it, but the pustule could be easily detached from the leaf and the insect was then discovered beneath it. No fructification was observed, and the fungus became known as the "brown mealy-wing fungus."

According to Webber, the hyphae of the fungus develop in the body of the insect, burst out round the margin of the scale, and gradually grow up over it. In an early stage, the fungus forms a compact brown layer round the edge of the insect, but as it develops it covers the scale completely in a dense, hard, smooth stroma. The size of the stroma varies according to the size of the insect attacked, attaining a diameter of 2 mm. and

a thickness of 175–200 $\mu$  (Plate I B). The colour of the stroma is commonly seal-brown, with a shade of chestnut, becoming slightly darker with age, the individual hyphae being light-brown, very tortuous, and only slightly branched.

Webber stated that a hypothallus extended from the base of the stroma over the leaf, forming a compact membrane near the stroma, but becoming dispersed into separate filaments outwards. He traced these filaments for a distance of 13 mm. from the stroma. The hyphae of the hypothallus were colourless, sparingly branched, usually continuous, with only an occasional septum, and 5–7 $\mu$  diameter. In places where they were somewhat massed together, they become pale brown, similar in colour to the individual hyphae of the stroma. In his summary, Webber described the hypothallus as a silvery white mycelium.

Parkin, in his *Fungi parasitic on Scale Insects*, referred to three collections gathered in Ceylon on *Aleyrodes*, which appeared to resemble Webber's brown mealy-wing fungus. One of these was on *Memecylon capitellatum*, in association with *Aschersonia placenta*; another on *Jasminum Sambac*; and a third on *Calophyllum Walkeri*. Parkin described the pustules of the second collection as rich brown, with a white membranous border from which hyphae radiated out to some extent over the leaf. He also described another gathering, on *Flemingia strobilifera*, in which the leaves bore normal *Aschersonia* stromata, old arrested *Aschersonia* stromata, and others which resembled Webber's fungus; and suggested the possibility that Webber's fungus was a sterile resting form of the *Aschersonia*, though he noted that Webber's account of the development and spread of the brown fungus did not favour such a view.

In 1908, Fawcett summarised Webber's and Parkin's accounts, and stated that in Florida there was no evidence of any connection between the sterile brown fungus and the *Aschersonias* parasitic on *Aleyrodes citri*. Cultures of the latter had never shown any tendency to develop the brown sterile form of the brown fungus. Attempts to obtain cultures of the brown fungus had been unsuccessful. The only growth under artificial conditions had occurred in one case in which stromata of the brown fungus were placed close to a drop of agar in a hanging drop culture, with the result that short tortuous hyphae grew out from the edge of the stroma. In a supplementary note, Fawcett stated that what appeared to be the spores of the brown fungus had been discovered.

The latter announcement was amplified by Fawcett two years later, principally in two accounts from which the following particulars are taken.

From the margin of the chocolate-brown stroma, colourless

thick-walled hyphae extend over the leaf. Later, usually in the summer or autumn, these hyphae grow out, long and colourless, extending not only over the under surface, where the stromata are situated, but over the margin and along the upper surface. The hyphae on the upper surface bear short lateral branches, on which sporodochia,  $60-90\mu$  diameter, are borne. These sporodochia (Plate I A, fig. 1) are aggregations of conidia-like, inflated, spherical cells,  $12-18\mu$  in diameter. From near the place of attachment of the sporodochium there radiate three to five hypha-like appendages,  $150-200\mu$  long,  $6-8\mu$  diameter, with one to three septa.

According to Fawcett, the entire aggregation of spherical cells and appendages usually remains united and functions as a spore. In hanging drop cultures of water or 5 per cent. glucose, they produced hyphae identical with those which compose the brown stromata. The first hyphae grew from the ends of the appendages. Experiments demonstrated that the larvae of the white fly could be infected by means of these sporodochia, and the brown stromata produced.

When the sporodochia are abundant, the upper surface of the leaf appears as if powdered with a reddish-brown dust. Fawcett states that this condition was first noticed in 1905. It may be noted that they are present on the specimen of the brown fungus sent by Webber to Parkin, which was collected at Manatee, Florida, March 1896. Perhaps the reason why they were ignored prior to 1908 is simply that no one expected that the stromata would have such a fructification, or that it would occur at such a distance from them.

Fawcett named the fungus, *Aegerita Webberi*. It would appear doubtful whether it is to be regarded as a true conidial fructification, as the apparent conidia do not become free. Fawcett suggested a relationship to the "*Hypochnaceae*" on account of the form of the hyphae, but the latter have no marked characters. The fungus has no resemblance to any stage or part of a *Meliola*, or Sooty Mould.

Numerous specimens of brown sterile stromata have been collected in Ceylon, and I have others from New Zealand, as well as specimens from Florida. On the characters of the stromata, it appeared probable that the forms from these three countries were different species; but the sporodochia in each are identical and they must all be referred to *Aegerita Webberi*, on the available evidence.

## FORM FROM FLORIDA.

Of this I have specimens which were sent by Webber to Parkin, and others kindly forwarded to me by Professor H. S. Fawcett. Both collections are on *Aleyrodes citri* on orange.

The stromata are rufous brown to rather light purple-brown, often mottled, glabrous, or minutely pruinose at first, flattened pulvinate or almost plane, more or less oval, up to 2 mm. long and 1.5 mm. wide, with a narrow, brown, fibrillose margin. They are frequently depressed in the centre, and often ring-shaped, not completely concealing the insect.

The hyphae which radiate from the stroma over the surface of the leaf are pale brown, 4–8 $\mu$  diameter, moderately thick-walled, somewhat flexuose, interlacing and sometimes fusing laterally in strands of three or four, but not forming a continuous film except at the edge of the stroma. These coarse hyphae run practically singly over the leaf and are not visible until the leaf is examined under a one inch objective.

Sporodochia occur along the hyphae on the lower surface of the leaf, but chiefly on the upper surface, where they may be massed in dense patches. Under such patches the hyphae are coalescent in a rather close network, the upper hyphae of which are brown, thin-walled, septate, up to 12 $\mu$  diameter, and more or less straight, while the lower are flexuose, hyaline, and united into a continuous film.

The scale persists beneath the stroma and is readily separable from it, the stroma being a thin layer, convex above, but concave below, following the shape of the scale. It is evidently hyphal, or plectenchymatous, except in the external layer, where the hyphae are fused together and appear in section as a parenchymatous cortical layer, one cell deep. The hyphae of the stroma are 3–8 $\mu$  diameter, pale brown, intertwined, and thin-walled. The stroma is not sclerotoid, nor can it be styled hard. In section it appears yellow-brown.

## FORM FROM CEYLON.

The Ceylon specimens, in those collections in which the host insect can be ascertained, occur, as a rule, on *Aleyrodes*, but sometimes on *Aspidiotus*. In very many cases, however, the host insect cannot be determined, as all the insects on the leaves have been attacked and no remains of them persist in the stromata. Parkin noted, in the case of his specimen on *Calophyllum*, that the brown pustules were not proved to be on scale insects. Proof, by discovery of the insect, is impossible in most cases, as the insect has been completely destroyed by the fungus. It is only rarely, and in young stromata, that



remains of the scale are to be found beneath the stroma. In this respect, the Ceylon fungus differs remarkably from the Florida form, but the difference may be due in part to differences in the species of insects attacked. It is possible that the scales of *Aleyrodes citri* and *A. nubifera* may be more resistant to the action of the fungus than those of the Ceylon insects.

The full-grown stromata are purple-brown, glabrous, smooth, convex or scutate, up to 2 mm. diameter, with a narrow, white or pale brown, fibrillose margin. Old specimens may have a recurved margin. In some cases, coarse hyphae, hyaline or very pale brown, radiate in strands from the stroma, but not regularly all round. More generally, however, a continuous hyaline film spreads from the stromata over the leaf, sometimes covering the whole of the lower surface. When dry, this film often separates from the leaf in large patches.

The hyphae which radiate from the stroma, in the first case, are  $2-6\mu$  diameter, regular, rarely flexuose, fusing laterally. Many of these hyphae are solid; others are thick-walled, septate, and have granular contents. As a rule, they form a layer only one hypha thick. The edges of these hyphae often appear ragged, owing to the development of a marginal film.

The hyaline film consists of a close network of stout hyphae running in all directions, the interspaces being filled by an amorphous membrane. The main hyphae are identical with those already described, but the branches between them are more flexuose and intertwined, and tend to lose their outline and become merged in the hyaline membrane.

Sporodochia are not common on the Ceylon specimens. They occur scattered over the film on the lower surface of the leaf.

The stromata are up to 0.5 mm. thick in the centre. In section, viewed macroscopically, they show a dense, dark purple-brown, peripheral zone, followed by a brownish white tissue which becomes looser towards the base. By transmitted light, the peripheral zone appears pale brown, with a narrow, dark brown, limiting zone internally, while the remainder of the stroma appears hyaline. The peripheral zone occupies about one half the thickness of the stroma, and is parenchymatous, displaying in section polygonal or rounded "cells," up to  $8\mu$  diameter, or elongated "cells," parallel to the periphery, about  $16 \times 6\mu$ . The lower hyaline half of the stroma consists of loosely intertwined hyphae with large numbers of irregular masses of calcium oxalate. The hyphae of the stroma are up to  $10\mu$  diameter, thin-walled, pale brown to almost hyaline, and irregularly contorted. The stromata are not sclerotioid.

In a specimen on *Aleyrodes* on *Jasminum Sambac*, the stromata are young, pale purple-brown, and do not differ

greatly in appearance from the Florida form. But they do not contain any remains of the insect. In a collection of similar young specimens on *Turpinia*, the insect is present beneath the stromata.

In another specimen, on an *Aleyrodes* on *Sarcococca prunifomis*, Hakgala, May 1910, the stromata are moderately thick, but the tissue is composed merely of loose hyphae which do not form a pseudoparenchyma, and the stromata are densely filled throughout with irregular masses of calcium oxalate.

A gathering on *Aspidiotus* on *Psychotria Thwaitesii*, Hakgala, May 1910, contains several abnormal forms. Some stromata are normal; others have a loose, strigose mass of pale brown hyphae at one side; while others consist entirely of a loose, pulvinate, strigose mass of hyphae. These hyphae are pale yellow-brown, thin-walled, septate, like the stouter hyphae in the normal narrow border of the stroma. The same abnormalities occur in specimens on *Allophylus zeylanicus*, Hakgala, May 1912, and on *Pavetta indica*, Hakgala, May 1912.

The Ceylon form differs from the Florida form in its thicker stromata, the parenchymatous inner tissue, and the membranous film extending over the leaf. As regards the latter, the Florida form is probably variable, since Webber referred to the hypothallus as a silvery white mycelium; and the parenchymatous tissue is merely an extension of the single parenchymatous outer layer of the Florida form. All the differences may be ascribed to a more advanced development of the fungus under Ceylon conditions.

#### FORM FROM NEW ZEALAND.

Specimens from New Zealand, on an undetermined scale on *Melicytus ramiflorus* Forst., have been kindly forwarded by Mr G. H. Cunningham, who informs me that the fungus appears to be common in the forests of that country. It was collected in New Zealand by Colenso, and there are specimens in Herb. Kew, labelled "*Aschersonia*. New Zd., Colenso 3804 a. *Aschersonia zeylanica* Berk." in Berkeley's handwriting. The name was not published.

The stromata are dark purple-brown, convex, rugose, up to 2 mm. diameter, surrounded by a brown, strigose layer of coarse hyphae radiating from the stromata over the leaf and forming a byssoid film which becomes paler outwards.

The film is composed of two layers. The lower layer consists of hyaline hyphae, 4-6 $\mu$  diameter, usually thick-walled, much curved and interlaced and forming a continuous sheet. Over this runs a network of pale brown, thick-walled, septate, regular hyphae, up to 8 $\mu$  diameter.

Sporodochia occur in dense clusters along the margin of the leaf on the lower surface (on which the stromata are situated) and may extend over to the upper surface.

The stromata are up to 0.5 mm. thick. The base is flat, as in the Ceylon form, and no trace of the insect persists beneath it. In section, viewed macroscopically, the stroma shows a dense, dark purple, subtranslucent, peripheral zone about  $125\mu$  thick, followed by a pale brownish region which becomes looser and whiter in the centre of the base. By transmitted light, the superficial layer is brown, but the remainder of the peripheral zone is hyaline; this is followed by a yellow-brown region, hyaline at the base. Except for a small region in the centre of the base the whole of the stroma is parenchymatous, with small cells,  $6-8\mu$  diameter, or oval or elongated cells, up to  $20 \times 10\mu$ . The cells in the peripheral zone are usually small and polygonal, the larger cells occurring in the lower half of the stroma, with their long axes parallel to the periphery, the hyphal nature of the stroma being thus more clearly indicated in that region. The cells have strongly refractive contents, and on that account the structure of the stroma is somewhat obscure when the section is examined in water, but the limits of the cells become clear when the section is mounted in chloroform or xylol.

The hyphae of the stroma are irregular,  $4-8\mu$  diameter, sometimes expanding to  $12\mu$ , thin-walled, septate. A few thick-walled hyphae,  $2\mu$  diameter, occur towards the periphery. In a small region in the centre of the base the hyphae are loosely intertwined and mingled with crystals of calcium oxalate. All the hyphae have solid contents in discontinuous, ovoid or irregular masses, free from the wall of the hypha. These bodies do not dissolve in alcohol, and are unaffected by chloroform or xylol.

The stromata in the *Colenso* specimen in Herb. Kew are similar to the above in external appearance, and show the dark peripheral zone in section. But the specimen examined was hollow in the centre, and contained an abundance of masses of calcium oxalate. The solid cell contents occurred only sparingly here and there, most of the cells lacking them.

The New Zealand form differs from the Ceylon form in the more parenchymatous stroma and the stouter film extending over the leaf. Here again the differences appear to be due merely to a stronger development of the fungus.

#### COMPARISON OF THE STROMATA.

In the specimens from Florida, the stromata are parenchymatous only in a single external layer; in the Ceylon form, they are parenchymatous for half their thickness; while in the New Zealand form they are parenchymatous down to the base. In

all cases the hyphae of the stromata are thin-walled, and the stromata are not sclerotoid or hard. In the New Zealand form, ovoid or irregular masses occur within the hyphae of the stroma.

In the Florida form, stout hyphae spread separately from the stromata over the leaf, uniting into a continuous film where the sporodochia are produced; in the Ceylon form, the spreading hyphae unite from the first into a hyaline film surrounding the stromata and covering the under surface of the leaf; in the New Zealand form a stouter byssoid film is produced, consisting of a hyaline continuous layer overlaid by a network of stout, pale brown hyphae.

In the Florida form, the scale persists beneath the stroma, but in both the Ceylon and the New Zealand forms no trace of the insect remains under the fully developed stroma.

#### THE SPORODOCHIA.

In the Florida form, the sporodochia occur chiefly in dense groups on the upper surface of the leaf, but they may also occur on the lower surface in similar groups near the margin or scattered along the repent hyphae. In the Ceylon collections, sporodochia are not numerous, and they occur on the lower surface scattered over the hyaline film. In the New Zealand form, the sporodochia occur chiefly clustered along the margin on the lower surface of the leaf, but they may extend over to the upper surface. In all cases the stromata are on the lower surface of the leaf.

The colour of the sporodochia varies slightly between the different forms. Those in the New Zealand specimens are dark rufous brown. In the Ceylon examples they are pale cinnamon brown, and in the Florida specimens rufous brown.

The sporodochium, about 0.1 mm. diameter, is a globose aggregate of spherical or ellipsoid cells, from which radiate three to six, usually four, appendages or setae (Plate I A, fig. 1). The conidia-like cells are thin-walled, hyaline or pale brown, smooth, either 12-16  $\mu$  diameter, or 13-20  $\times$  10-16  $\mu$ . They are in short, branching, curved chains (Plate I A, fig. 6), and do not separate from each other or from the sporodochium. The setae are pale brown or almost hyaline, cylindrical, two to three septate, equal or slightly attenuated upwards, with an obtuse apex 50-180  $\mu$  long, 6-9  $\mu$  diameter, usually thin-walled, but sometimes with a wall 1  $\mu$  thick (Plate I A, fig. 3). In some setae the basal cell is slightly inflated, and others may be slightly constricted at the septa, but in general they are uniformly cylindrical. The apex of the immature seta is often acuminate, and in one instance a spear-shaped tip was observed (Plate I A, fig. 5).

The sporodochia arise from the repent hyphae singly, on short, lax, flexuose, septate stalks, 4–5 $\mu$  diameter. At the apex, the stalk expands into a pyriform head, about 30 $\mu$  high, and 12 $\mu$  diameter above, which gives rise to chains of oval or globose cells, or to setae, at any point (Plate I A, fig. 2). The chains of cells are usually irregularly curved, and branch repeatedly, the branches spreading, or curving parallel to the original chain, or even curving backwards. The chains are consequently variously intertwined, and do not separate when the sporodochium is submitted to pressure under a cover glass. If, however, the cover glass is moved from side to side, especially after treatment of the sporodochium with lactic acid, the apex of the stalk and the initial cells break up, and the sporodochium is resolved into short, curved chains of cells.

The setae frequently arise in pairs (Plate I A, fig. 4). They may originate from a common basal cell attached to the apex of the stalk, or as lateral branches in a chain of cells. They originate in the sporodochium, not beneath it.

#### CULTURES.

Fawcett, in 1908, stated in the body of his paper, that attempts to obtain cultures of the brown fungus had been unsuccessful; but in an appendix he recorded that what appeared to be the spores of the fungus (*i.e.* the sporodochia) had been discovered, and these were germinating in hanging drop cultures of sugar solutions.

In his paper of 1910, Fawcett wrote that "These *Aegerita* sporodochia, when germinated in hanging drop cultures of sterile water and in 5 per cent. glucose solution, were seen to produce hyphae identical with those which compose the brown stromata on the white fly larvae. When germinating, the first hyphae grow out either from the sporodochia or from the ends of the appendages. These branch rather sparingly, but in a few days, in 5 per cent. glucose solution, form a network by the intercrossing of the branches."

Specimens were collected at Hakgala on an *Aleyrodes* on *Turpinia pomifera* in September 1923. The stromata were young, having evidently been formed during recent wet weather after the dry season which prevails during the first half of the year on that side of the hills. The scale insects still persisted beneath the stromata, and hyphae were beginning to radiate from the stromata over the leaf. Only on one leaf was it possible to find sporodochia, and these, though full size, were still white.

Sporodochia were picked off the leaf with a sterile needle, and placed in hanging drops of Raulin-maize meal agar in damp cells. Growth of hyphae was observed after two days, but in no

instance did it proceed from the cells of the sporodochium. In one instance, the mycelium arose from a *Cladosporium* spore, and in another, from a *Pestalozzia* spore, entangled among the cells of the sporodochium. The sporodochia in the hanging drops were treated with lactic acid, and then broken up into their component chains of cells; in no case could it be seen that any growth had occurred from the globose cells or from the setae. A yeast developed round the sporodochium in one hanging drop.

Sporodochia were also placed on slants of maize-meal agar, maize-meal agar plus Raulin's fluid, and neutral Raulin agar, but in all cases the tubes produced either a *Penicillium*, or a *Pestalozzia*, or both.

Others were sown, singly, in flasks of neutral Raulin's fluid, and Naegeli's solution, No. 3, with cane sugar, three of each series. In each series no growth occurred in one flask, while another in each series developed *Penicillium* and *Pestalozzia* respectively. The third flask in the neutral Raulin series developed a dense, submerged, dark green mycelium; this ultimately formed a densely tomentose layer at the surface, with oval conidia, from  $4 \times 3\mu$  to  $16 \times 5\mu$ ; and transfers of these conidia to bean agar produced compact, green patches of a *Cladosporium*. In the third flask in the Naegeli series, a red-brown, submerged mass of mycelium was produced, which proved to be an unidentified laboratory contamination.

In January 1924, after the monsoon rains, cultures of sporodochia from the same tree were again attempted. Six flasks of Naegeli's fluid were inoculated with a single sporodochium each. In one of these no development occurred; the others developed *Pestalozzia*, *Cladosporium*, *Fusarium*, and other Hyphomycetes.

Attempts were also made to obtain cultures from the stromata. Horizontal sections of these were cut with a sterile knife, the outer layer being discarded, and the base left adherent to the leaf. These were placed in tubes of maize-meal agar, and neutral Raulin agar, and flasks of Raulin's and Naegeli's fluids. In one flask of Raulin's fluid, no growth occurred, while in all the other cases *Penicillium*, *Cladosporium*, and *Pestalozzia* developed.

In another series, the stromata were brushed over with alcohol, all the hyphae which spread from them over the leaf being either broken by the brush or cut with a sterile knife. They were then detached from the leaf and the outer layer shaved off. One lot was cut into small pieces; another was ground up in a glass mortar with sterile sand. The latter process was not very successful, the stromata being too soft and adhesive to break up into small fragments. Both lots were then plated out separately in neutral Raulin agar. Growths

were obtained of *Penicillium*, *Pestalozzia*, *Botrytis*, and *Cladosporium*, but no growth could be detected from the majority of the fragments of the stromata.

In one instance, however, a development of hyphae was noted from one piece of stroma, and this was transferred to a tube of neutral Raulin agar. Growth was slow, a compact, strongly convoluted stroma, about 2.5 cm. long, 1.5 cm. broad, and 7 mm. high, being produced in four months. The stroma was minutely tomentose, black-brown, with a reddish, or purplish, fuscous, tomentose surface layer. It proved to be hollow, forming an irregular shell, about 1 mm. thick. Its advancing margin was entirely submerged, about 1 mm. broad, and composed of radiating hyphae, but the remainder of the stroma was plectenchymatous, and in its subsequent growth had been raised above the surface of the agar in irregular folds.

The hyphae of the advancing margin were pale purple-brown, fairly regular, septate,  $3\mu$  diameter, sometimes with swellings at the septa, with other hyphae, closely septate and composed of moniliform chains of cells, up to  $10\mu$  diameter. The stroma was yellow-brown or pale purple-brown in section by transmitted light, and consisted of cells up to  $16 \times 10\mu$ . In the old culture, the contents of these cells were strongly refractive. The superficial layer consisted of rigid, septate hyphae, up to  $30\text{--}80\mu$  high,  $4\mu$  diameter, and globose cells, about  $8\mu$  diameter, in short chains or irregular clusters. The latter cells resembled the segments of the moniliform hyphae found in the advancing margin, while the short, rigid hyphae bore a considerable resemblance to the setae of the *Aegerita* sporodochia. The stroma was evidently built up by the fusion of the two kinds of hyphae.

When pieces of the stroma in the foregoing culture were transferred to slants of Naegeli agar, a similar stroma was produced, but the colour was greenish black. The structure was the same, but the seta-like hyphae were shorter, about  $30\mu$  long.

On transfers to maize meal slants, no superficial stroma was formed. The mycelium was greenish black, submerged, with a slight grey superficial layer, and on the surface of this, small fuscous pycnidia, 0.3 mm. diameter, with very minute spores, were produced.

It was unfortunately not possible to continue these investigations further. The stromata obtained in culture were possibly those of *Aegerita Webberi*, though probably not pure. The stromata of *Aegerita* are frequently parasitised by pycnidial fungi, and it may have happened that one of these was present in the cultures, but developed fructifications only on the maize meal. The cells of the stroma resembled those of the *Aegerita*

stroma in their refractive contents, and in their general shape and size, though little weight can be placed on the latter. Further, the short rigid hyphae on the exterior of the stroma resembled the setae of the *Aegerita* sporodochium. That the shape of the stroma differed from that of the *Aegerita* stroma is perhaps immaterial; the latter develops at the expense of a limited supply of food, viz., that contained in a single scale insect, while the former had a food supply available over a large area. On the other hand, the stromata obtained in culture did not produce repent hyphae extending indefinitely from the margin, even when they had come in contact with the sides of the tube.

Some apology is needed for presenting these incomplete results, but it is possible that future workers may find them of some use. The difficulties of obtaining pure cultures of *Aegerita Webberi* are obvious. The cells of the sporodochia do not separate from one another naturally, and consequently attempts must be made to start cultures from entire sporodochia, which may have spores of other fungi entangled in them. Similarly, the stromata in their development may overgrow and include fungus spores, and growth may occur from these when fragments of the stroma are used. Foreign spores may sometimes be seen embedded in the surface layers of the stroma.

It might be suggested that the stromata of *Aegerita Webberi* are not pure growths of a single fungus, but a mixture, the barren stromata being produced by the growth of a super-parasite on an entomogenous fungus. It is known that when the stromata of *Aschersonia placenta* are attacked by one species of *Cladosporium*, they become more discoid, greenish yellow and corky, quite different in appearance, colour, and texture from the normal stromata; the pycnidia are not suppressed, but the spores and paraphyses are imperfectly developed and form a brown lining to the pycnidium. Again, in a collection of the same species, parasitised by a *Comiothyrium*, the *Aschersonia* stromata are hard, discoid, and purple-brown, the pycnidia, spores, and paraphyses being in the same condition as in the foregoing. Such specimens, if gathered alone, could not be classified correctly, and might be taken to be new species, as indeed has happened in the case of similarly parasitised entomogenous fungi, though the abnormal condition of the contents of the pycnidia should give cause for hesitation.

It does not, however, seem probable that the *Aegerita* stroma is due to parasitism of this nature. In other entomogenous fungi, it is usual to find normal specimens in company with the parasitised stromata. On the other hand, the stromata of *Aegerita Webberi* occur in large numbers, all of the same



structure and appearance. They do occur with other entomogenous fungi occasionally, but the majority of the collections made in Ceylon consist solely of *Aegerita stromata*, no other stromata being present of which they might be considered parasitised examples.

Nor does it appear probable that the stromata are formed from parasitised sporodochia. In that case, one would expect to find examples in which the insect bore only repent hyphae and sporodochia. I have one specimen in which mycelium bearing sporodochia spreads from a scale insect which does not bear an *Aegerita* stroma; but it is an exceptional case, and the insect is wedged beneath a scale leaf of *Juniperus*, where a normal *Aegerita* stroma could not be formed. The sporodochia of *Aegerita Webberi*, in general, do not occur on the scale insects, but on repent hyphae which extend from the stromata over the leaf.

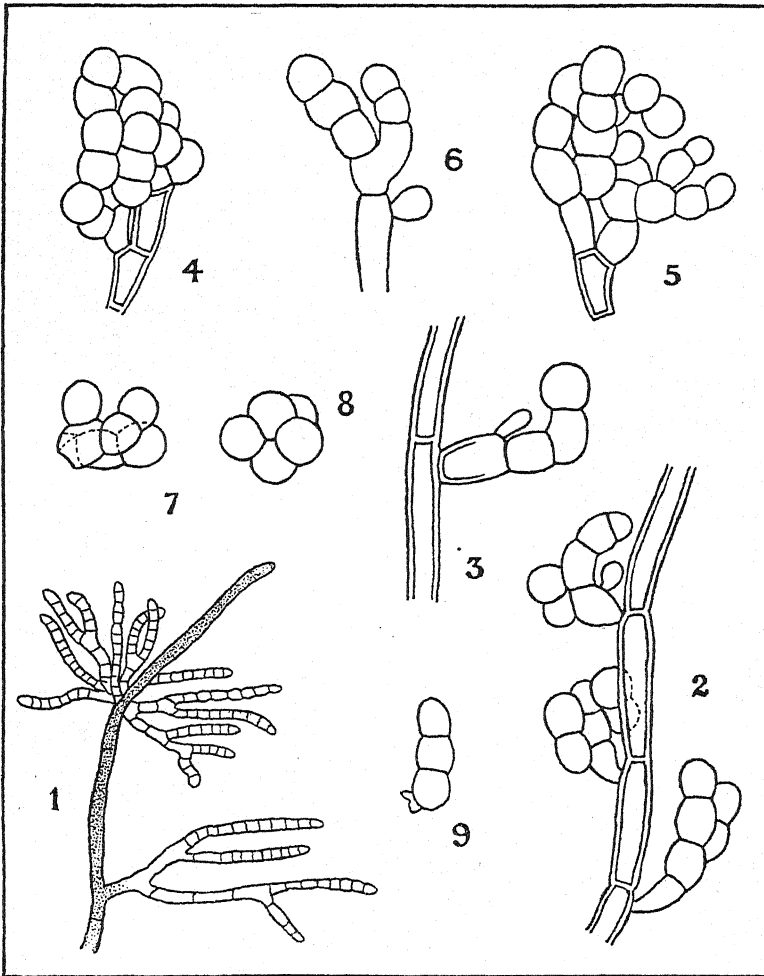
Fawcett's hanging drop cultures do not appear to afford conclusive evidence of the germination of the sporodochia. Similar growths of mycelium were obtained in the present experiments, but it was determined that they did not arise from the cells of the sporodochium. The only conclusive evidence would be the production of sporodochia, or stromata, identical in structure with those of *Aegerita Webberi*.

The failure to obtain pure cultures from the sporodochia or stromata throws considerable doubt on the validity of the accepted method of distributing this fungus for the purpose of destroying *Aleyrodes*. It is claimed that successful propagation is obtained by grinding up in water leaves which bear the fungus, and spraying trees infested with *Aleyrodes* with the mixture. This argues a ready germination of the sporodochia, or a rapid growth from fragments of the stroma, both of which are contrary to the experience of everyone who has tried to cultivate this fungus.

#### CONIDIAL FORMS OF *Septobasidium*.

In *Bull. Soc. Myc. France*, XL, p. 29 (1924), Patouillard described *Septobasidium lanosum*, parasitic on coccids on *Citrus decumana* (Pumelo) in Tonkin. The fungus forms extensive resupinate patches, dark brown (ombre-brun) with a reddish sheen, woolly, with a narrow grey margin. Its context is soft and floccose, composed of erect, brown, branching hyphae, fasciculate in bundles of six to ten, not interwoven into a superficial sheet. The probasidia are hyaline, globose, scarcely pedicellate, 12-15 $\mu$  diameter, situated on the upper part of the erect hyphae. Above the point of insertion of the probasidia, the hyphae continue as circinate branches, 4-6 $\mu$  diameter and of varying length.

Mixed with the hyphae which bear the probasidia, and identical with them in appearance, though not coiled in tendrils at the ends of the branches, are a large number of other hyphae which bear short, hyaline, lateral branches, simple or forked,



Text-fig. 1. Conidial *Septobasidium*; figures 2-9,  $\times 600$ .

solitary or in tufts,  $30-75\mu$  long, thin-walled, capable of dividing by transverse septa into a large number of segments,  $4-5\mu$  long, which can separate ("susceptibles de se séparer") from one another and so form an abundance of conidia. After the appear-

ance of the septa, the lateral branch is constricted at the septa and appears moniliform, and the segments or conidia are ovoid.

Patouillard did not find probasidia and conidia on the same hypha, though they occur on similar hyphae in the same individual specimen. He records that similar conidia occur in *Septobasidium albidum*, which is also found on coccids on *Citrus*; but in the latter species the conidial specimens are almost always destitute of probasidia.

Part of Patouillard's figure is reproduced herewith (Text-fig. 1). It will be seen that the lateral branches, in general, terminate in a cell which increases in width upwards and bears chains of conidia at the apex.

A similar fungus, on a scale insect on tea from Yaizu, Province Suruga, Formosa, has been forwarded to me by Mr K. Hara.

It forms loosely woolly, dark purple-brown tufts, 0.5 mm. thick, oval, a few millimetres long and broad, which become confluent in large patches. The hyphae are crowded, more or less erect, flexuose and intertwined, reddish brown or dark yellow-brown, paler at the apex, septate, thick-walled, minutely rough, 6–8 $\mu$  diameter, sparingly branched. The hypha may be attenuated above, with an acute apex, or it may terminate in a group of spore-like bodies.

The spore groups arise from the hypha below a septum, or at the apex, or on short lateral branches, up to 50 $\mu$  long, which are two to three septate. In the first case a curved basal cell, expanding upwards, arises from the hypha, and bears two or more short chains of cells, usually with three cells in a chain. These chains resemble three-celled, cylindric spores, constricted at the septa and usually curved, about 24  $\times$  10 $\mu$ . They do not, however, become free, but give rise to similar chains either laterally or apically, forming a loose head, either globose or oval, up to 40  $\times$  25 $\mu$ . The chains arise as a rule from the apex of the basal cell, but they may originate at any point on it. The short lateral branches terminate in similar clusters, but they may bear in addition chains of cells, each arising below a septum of the branch.

Some of the short chains may occasionally be found detached, but in that case fragments of another cell are attached at the base, showing that the chain has been broken off (Text-fig. 9). As a rule, the spore cluster, including the basal cell, is detached as a whole (Text-figs. 4, 5). The basal cell is usually thick-walled.

The chains are not united into a solid mass, and consequently the spore group does not resemble the conidium of *Septosporium*.

Text-figs. 2 and 3 show the clusters of chains developing laterally on a hypha, and Text-fig. 6 a developing apical cluster.

Text-figs. 4 and 5 show two detached clusters, and Text-fig. 9 a single chain. Text-figs. 7 and 8 give lateral and apical views respectively of a small detached cluster.

Probasidia have not been observed in this specimen, but from its general structure it would appear probable that it is a conidial form of a *Septobasidium*.

The resemblances between the foregoing "conidial" forms and the sporodochia of *Aegerita Webberi* are scarcely sufficiently marked to justify the conclusion that the latter is a form of a *Septobasidium*. Nevertheless, the fact that such forms do occur in *Septobasidium* suggests that possibility, and consequently it has been considered worth while to refer to them here. No links have been found between *Aegerita Webberi* and any Ceylon species of *Septobasidium*. *Septobasidium Thwaitesii* forms thin, flat, glabrous stromata, purple-brown in colour, which are circular or oval, with a byssoid margin, at first, and subsequently coalesce into a continuous sheet. But these stromata are composed of loose hyphae internally, and do not exhibit any trace of the parenchymatous structure of *Aegerita Webberi*.

#### FUNGI PARASITIC ON *Aegerita Webberi*.

Morrill and Back recorded the occurrence of a parasitic fungus on *Aegerita Webberi* in Florida, which was referred to *Coniothyrium*. They stated that it formed a dense, dark greenish, hard growth over the pustule of the brown fungus, and presented a surface roughened by numerous pustular elevations. I have specimens from Fawcett which agree exactly with Morrill and Back's figure, the pustular elevations being pycnidia.

A similar fungus occurs on *Aegerita Webberi* in Ceylon, and has been described by the writer as *Sirosphaera chlorostoma* (*Trans. Brit. Myc. Soc.* VIII, p. 208 (1923)). In the original description it was stated that the pycnidia bore scattered erect hairs, up to  $44\mu$  high,  $6\mu$  diameter below, tapering upwards. A re-examination of the Ceylon examples in comparison with specimens from Florida shows that there are, in addition, slender yellow acicular setae in spreading fascicles: these are apparently deterrentive and were overlooked at the previous examination.

A Florida specimen of *Aegerita Webberi* from Professor H. S. Fawcett bears good examples of this *Sirosphaera*. The pycnidia are conoid, about 0.2 mm. diameter, clustered, often in a ring round the edge of the *Aegerita* stroma. The conidia are brown in mass, pale fuscous individually, narrow-oval or oblong-oval,  $2-4 \times 1.5-2\mu$ , or subglobose,  $1.5-2.5\mu$  diameter. The pycnidia bear short scattered hairs, but they differ from the Ceylon form in the copious development of the acicular setae. These latter

occur in spreading fascicles, not only on the pycnidia, but also on the exposed parts of the stroma, in such abundance that the whole fungus appears green. The individual setae are yellow, rigid, up to  $50\mu$  long,  $1.5\mu$  diameter at the base, attenuated upwards regularly to the acute apex.

The Florida form does not differ from the Ceylon form except in the more abundant development of the setae, and it is doubtless the same species.

A specimen of *Aegerita Webberi*, collected on *Psychotria* at Hakgala, March 1922, has the stroma blackened by minute, pulvinate, close-set or confluent sporodochia. These sporodochia are parenchymatous at the base, and the conidiophores, after a short base,  $8\mu$  high and  $2\mu$  diameter, are branched, with branches about  $6\mu$  long. These branches bear at their apices densely clustered, flask-shaped phialides,  $8\mu$  long. The conidia are hyaline, oval, continuous,  $2 \times 1\mu$ . This species appears to be a *Tubercularia*, which may be known as *Tubercularia epimyces*. From the stroma, hyphae radiate in a film over the leaf; these are fuscous,  $2\mu$  diameter, septate, thin-walled, slightly irregular, and united laterally into strands. On these hyphae are blackish-fuscous parenchymatous bodies, about  $50\mu$  diameter, which may be developing pycnidia; they bear irregularly conical fascicles of hyphae, or single, erect hyphae, up to  $30\mu$  high. The same fungus has also been found on stromata of *Torrubiella*.

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#### EXPLANATION OF PLATE.

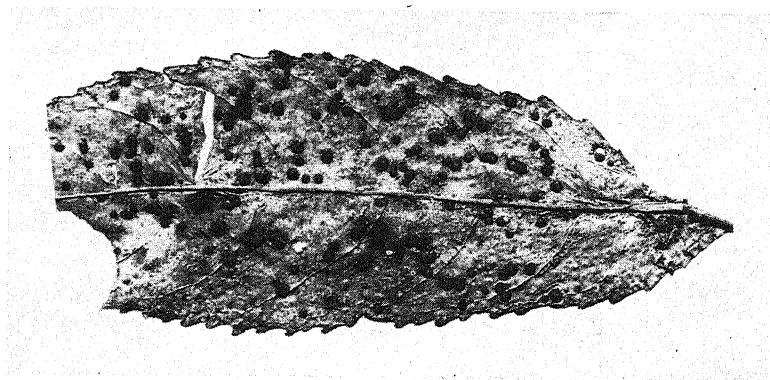
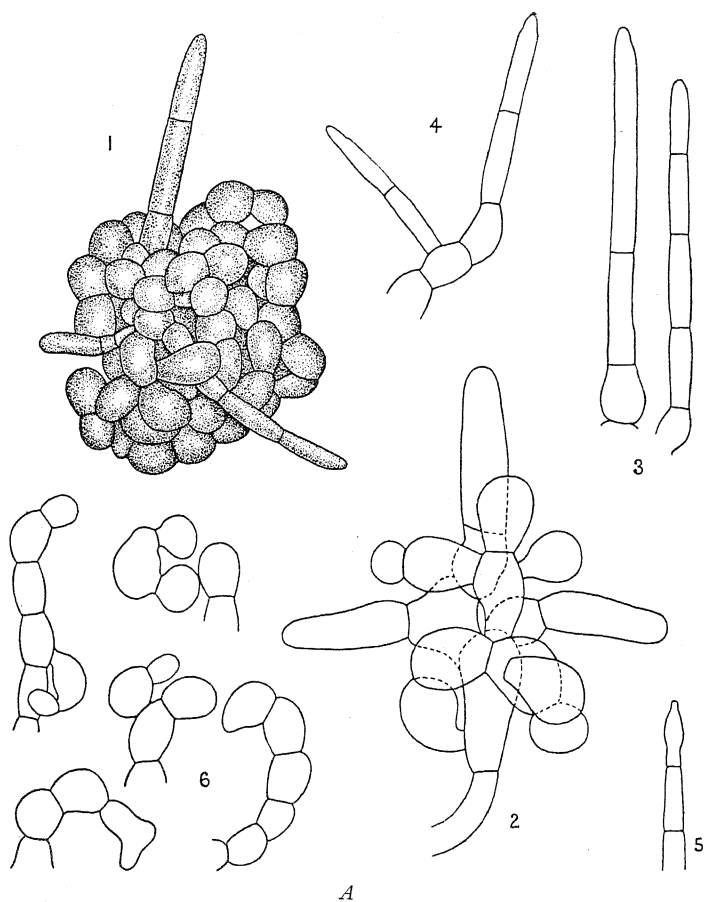
##### PLATE I.

##### A.

- Fig. 1. A sporodochium.  $\times 400$ .  
 Fig. 2. Early stage of formation of a sporodochium.  $\times 750$ .  
 Fig. 3. Setae.  $\times 400$ .  
 Fig. 4. Two setae arising from the same chain of cells.  $\times 400$ .  
 Fig. 5. Seta with an acuminate tip.  $\times 400$ .  
 Fig. 6. Chains of cells from a sporodochium.  $\times 400$ .

##### B.

*Aegerita Webberi*, specimen from New Zealand. Natural size.



B

*Aegerita Webberi*



## MATULA.

By T. Petch, B.A., B.Sc.

(With Plates II and III, and 2 Text-figs.)

IN *Fungi Cubenses*, Berkeley instituted a new genus, *Michenera* B. and C., with the characters, "Placentaeformis, disco ceraceo; sporis magnis limoniformibus longe pedicellatis." The type species, *Michenera artocreas* B. and C., was said to occur on dead trunks of trees in Cuba (Wright 262), and on Black Oak in the United States (Curtis 5609). It was from one-quarter to three-quarters of an inch across; margin raised, white tomentose; hymenium cracked, umber, resembling that of *Corticium ochroleucum*; spores, including the stem, .0025 inch long, without the stem .001. In the Introduction to the paper, Berkeley stated that "*Michenera*, with which we have long been acquainted as occurring in the United States, is peculiarly interesting in point of structure."

Later, in *The Fungi of Ceylon*, Berkeley and Broome described the genus *Artocreas*, "Receptaculum commune distinctum; hymenium planum e sporis magnis pulveraceum." The type species, *Artocreas poroniaeforme* B. and Br., Thwaites 309, was "erumpens; receptaculo hemisphaerico, pallide rufo; hymenio albedo; sporis globosis. Looking just like an imperfect *Crucibulum*. Spores globose, .0009 in diameter."

To the description of the Ceylon species, Berkeley and Broome added: "A species of the same very distinct genus occurs in the United States"; and they proceeded to describe the latter as "*Artocreas Micheneri* B. and C. Extus album tomentosum; hymenio rufo; sporis ovatis apiculatis pedicellatis (Nos. 3529, 3773). Pennsylvania, Michener. About  $\frac{1}{4}$  of an inch wide, or more by confluence; spores ovate, with a long obtuse apiculus, .0013 long without the apiculus."

It would appear that Berkeley had forgotten that he had already described this second species as *Michenera artocreas*. Against that supposition, it has been pointed out that Berkeley did not cite the same collection numbers for *Artocreas Micheneri* as for *Michenera artocreas*. But all doubt as to Berkeley's intention is removed by his note in the supplement to *The Fungi of Ceylon*, where it is stated (p. 83), "*Artocreas* B. and Br. It is necessary to remark that this is synonymous with *Michenera* B. and Curtis, and that *Artocreas* B. and C. (*sic*) is the same with *Michenera artocreas* B. and C., Cuban Fungi, No. 413." Thus Berkeley withdrew the genus *Artocreas*.

In 1888, Massee published an account of the Ceylon fungus *Artocreas poroniaeforme*, and instituted for it a new genus



*Matula*, which he placed in a new order of Gasteromycetes, Matuleae, intermediate between the Nidulariaceae and the Hymenogastraceae. According to Massee, the leading features of the plant were: "(1) a peridium closed above by an epiphragm until all differentiation is completed; (2) a gleba broken up into numerous cavities or loculi by dissepiments bearing basidia on their free surfaces." The basidia were described as "very primitive in structure, being slightly or not at all thickened at the apex, and producing usually a single spore which at first appears as an obovate terminal cell attached by a broad base. While the spores are still young and obovate they are set free by the total disappearance of the basidia, afterwards becoming spherical and increasing considerably in size."

The type species of the genus *Michenera* is *Michenera artocreas* B. and C. The type species of the genus *Artocreas* is *Artocreas poroniaeforme* B. and Br. Berkeley withdrew the latter genus, as being synonymous with *Michenera*. The Ceylon fungus consequently became *Michenera poroniaeformis*, though Berkeley did not publish that combination. When Massee decided that the Ceylon fungus was not co-generic with *Michenera artocreas*, he did not revive Berkeley's generic name *Artocreas*, but founded a new genus *Matula*.

It appears to have been generally assumed that the type of *Michenera artocreas* B. and C. is the United States specimen, Curtis 5609, and enquiries into the identity of *Michenera* have generally been based on the North American species. But the type, by Berkeley's citation, is Wright 262, of the second Wright collection, and the type locality is Cuba, not the United States.

In the modern school of nomenclature, type specimens and type localities are assigned the principal part in the determination of an author's idea of his species. Hence it is of the greatest importance that these should be quoted correctly. It has been a fairly common practice to claim North American localities as type localities of Cuban fungi, owing to the method adopted by Berkeley in the publication of *Fungi Cubenses*. He did not consider it necessary to give the locality, Cuba, but, after some of the species enumerated, he added, under habitat, a list of localities which are not Cuban. That is explained in the introduction, where Berkeley wrote: "The habitats appended to species already described are taken from specimens in our herbaria, or such as have passed through our hands." The paper shows that the restriction to species already described was not observed, for Berkeley added extra-Cuban habitats to some of his new species. It is most probable that, as in the case of *Michenera*, he had had these new species in his herbarium for some time, although he had not published descriptions.

It is also clear, from an examination of the specimens, that

in many cases the Cuban fungus is not the same as the North American one. Indeed, it would appear that many of these extra-Cuban localities were added from memory, or from macroscopic examination only. In such cases, where new species are concerned, the Cuban fungus should, by Berkeley's citation, be the type.

For example, *Xylaria clavulata* B. and C. was recorded without descriptions from Wright 312, with the synonym *Sphaeria clavulata* Schw., and the extra-Cuban localities, United States and Venezuela. But Wright 312 is an immature *Xylaria*, while *Sphaeria clavulata* Schw. is a *Cordyceps*. The two fungi are not related, but as *Sphaeria clavulata* antedates *Xylaria clavulata*, no difficulty arises.

Again, *Nectria diploa* B. and C. was described as a new species from Wright 606, and the extra-Cuban habitat, Carolina Inf., was added, with the number Car. Inf., 4029. The latter is not the same as the Cuban species, and the description does not fit it. The type specimen is Wright 606, the type locality Cuba, and the original description was evidently drawn up from the Cuban fungus. This example demonstrates that it is not correct to suppose that, because Berkeley had, or thought he had, a previous specimen from another locality, the latter is the type.

Consequently, in clearing up the problem of *Michenera* it is necessary to determine whether the North American fungus is identical with the Cuban species, and whether either is co-generic with the Ceylon species.

The Cuban specimen, Wright 262 in Herb. Paris, was examined by Patouillard, who published a description of it in 1891, and again in 1900. Except in a few minor details, Patouillard's description agrees with Lyman's account of the North American fungus quoted below. As, however, the specimen in Herb. Paris is only a co-type, there was still a possibility that North American and Cuban specimens might have been confused in the distribution. To decide the point, Miss Wakefield has kindly examined the type in Herb. Kew, and finds that Wright 262 has the same structure as Curtis 5609. Hence the North American and the Cuban specimens are at least co-generic, if not identical. The reservation is necessitated by the fact that we are dealing with conidial forms.

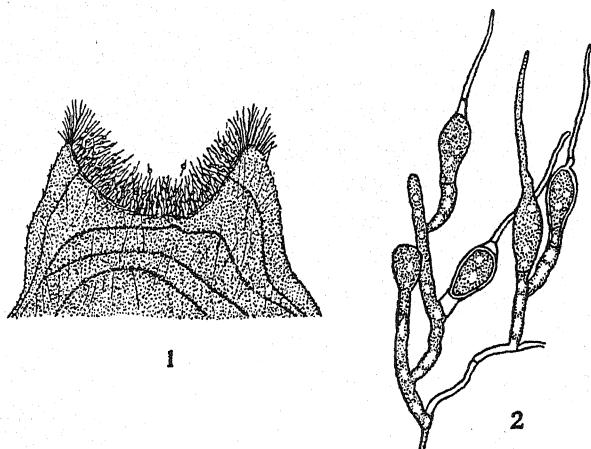
Patouillard noted that the cups of *Michenera artocreas* sometimes arose from the stroma of a *Corticium*, the hyphae of the two being identical, and continuous from one to the other. Hence he concluded that *Michenera* is a conidial form of a *Corticium*.

In the United States, *Michenera artocreas* usually grows in association with *Corticium subgiganteum* Berk. The *Corticium* occurs on the under side of the host branch, and the *Michenera*, when present, on the upper. On fallen branches, if the *Corticium*

happens to lie uppermost, *Michenera* cups may appear, arising directly from the hymenium, fused with the tissue of the *Corticium*, the hymenium of which overruns the bases and sides of the cups.

Pierce (1890) examined herbarium specimens of *Michenera artocreas*, and decided that it was a conidial stage of a *Corticium*, but that it was not related to the adjacent hymenia. Subsequently, Lyman (1907) re-investigated the question, and established that *Michenera artocreas* is a conidial form of *Corticium subgiganteum*. The details given here, concerning the North American fungus, are taken from Lyman's account.

*Corticium subgiganteum* is effused, rather tough, white, becoming corky and brownish when dry. The basidia are clavate,



Text-fig. 1. *Michenera artocreas* (after Lyman). 1. Vertical section of stroma.  
2. Conidiophores with developing conidia.

$40 \times 14.5\mu$ , and the basidiospores globose, apiculate,  $19 \times 14\mu$ . Massee referred it to *Peniophora*, but Lyman retained it in *Corticium*.

*Michenera artocreas*, according to Lyman, is cup-shaped, 3–20 mm. diameter, with white or brownish sides and a projecting rim, the cup being nearly filled with reddish brown spores adherent in a solid mass which becomes hard and cracked when dry. The cup may be solitary, or clustered, or more or less fused. In section they show three distinct regions—the sides and base of interwoven hyphae, the hymenium lining the cup, and the brown mass of spores which frequently fills the cup. The tissue of the cup is loose and spongy, and may show brownish layers which indicate periodic stoppages of growth.

“The first appearance of the cup is a small pyramidal growth

of hyphae, faintly brownish tinted on the exterior. A longitudinal section frequently reveals concentric brownish layers which mark the successive periods of growth in the intermittent development of the pyramid. The hymenium originates at the apex of the pyramid, where the hyphae begin to form spores and a brown spot appears. This spot becomes sunken, due to the continued upward growth of the surrounding sterile tissue to form the raised sides of the cup....The origin of the hymenium is superficial and its development is entirely gymnocarpic, for the cup makes its appearance by the continued upward growth of the tissue at the margin of the hymenium, not by the rupture of an originally closed vessel. The diameter of the fructification increases by the continued expansion of the hymenium, which develops not only in the bottom, but also up the sides to the margin of the cup."

The spores are terminal and solitary. They are ellipsoidal or lemon-shaped, with a persistent stalk and a long, slender, apical appendage. The ellipsoidal part is yellowish to deep reddish, thick-walled,  $12-20 \times 10-15\mu$ ; the stalk is about as long as the inflated part of the spore,  $3-4\mu$  diameter, paler in colour, and empty; the appendage is flexuose, hyaline, empty,  $1.5-2.5\mu$  diameter, and up to  $75\mu$  long. The sporophore may branch near the apex, so that a cymose cluster of spores is produced, while after the first spores have been formed, repeated basipetal branching occurs, either just at the bases of the spores or from lower parts of the sporophores. Numerous long, slender, hyaline paraphyses accompany the sporophores.

"The process of development of the spore is as follows. The end of a young sporophore, rich in protoplasm, enlarges to form the lemon-shaped spore. Meantime growth continues from the upper end of the enlargement to form the slender appendage or lash, which has about one half the diameter of the sporophore. As the spore approaches full size it is cut off from the sporophore by a septum some distance below the swollen portion, thus forming the stipe. The terminal appendage and the basal stipe become vacuolate and finally emptied of their protoplasm as it gradually concentrates in the body of the spore. The mature spore has a thick wall consisting of an episporium which is the wall of the original hypha and which also forms the walls of the stipe and the appendage, and an endospore which surrounds the inflated portion only. The endospore is formed during the period of concentration of the protoplasm. Therefore the stipe and the base of the appendage frequently show thin, curving, transverse septa, abandoned as the protoplasm shrinks inward, which belong to the endospore and mark the successive stages in the concentration of the spore's contents."

Lyman grew the *Michenera* form in pure culture from the basidiospores of *Corticium subgiganteum*, and from the conidia of *Michenera artocreas*, but he did not succeed in obtaining the *Corticium* form in culture.

*ARTOCREAS PORONIAEFORME* B. AND BR.

*Artocreas poroniaeforme* (Plate II, fig. 1) occurs sparingly in the jungle at Hakgala on fallen twigs, the identity of which has not been determined. During recent years it has occurred in abundance on twigs of *Cinnamomum Camphora* in the Hakgala Botanic Gardens, either on dead twigs attached to the tree, or more commonly on fallen twigs and prunings on the ground.

As usually collected, the fungus forms scattered hemispherical cups, up to 5 mm. diameter and 2.5 mm. high, erumpent from the branch. These are white, or slightly brownish on the exterior. At first the top is plane, slightly depressed below the rim of the cup, and usually reticulated with irregularly broken, brown lines. Such specimens have opened, and are about to liberate their spores, though the latter are not loose, but cemented together and to the wall of the cup in a continuous mass. The plane upper surface is not pulverulent, and the whole fungus is hard and compact when dry. During wet weather it absorbs moisture and expands slightly; the cementing substance softens, and the contents of the cup, which at this stage consist chiefly of spores, are washed out, or possibly removed by insects. When old, the cup is empty down to the base.

Vertical sections show that the wall of the cup is up to 0.5 mm. thick. It is hyaline in section, and consists of interwoven thick-walled hyphae, with lumina 1-2  $\mu$  diameter. The exterior may be slightly tomentose with free ends of hyphae, 2-4  $\mu$  diameter, thick-walled, or almost solid, irregularly curved sometimes bifurcating at the tips, sometimes dividing into a cluster of short, curved branches. In the context of the wall the limits of the hyphae are not discernible, their membranes being fused into a continuous mass, in which, on staining, the contents of the individual hyphae appear as widely separated, irregular lines. When a vertical section of the cup is placed in water, the wall swells and straightens out. As seen in section, the wall is horny when dry and subgelatinous when moist. In serial sections, the tissue of the wall is sometimes found to be interrupted in places by narrow gaps which extend completely through it horizontally and are filled by a loose web of hyphae. The hyphae bear clamp-connections. The wall is not present over the base of the cup.

Vertical sections through a cup which is filled with spores show a peculiar structure (Plate III, fig. 1). From the host

tissue at the base of the cup there arise more or less vertically oval bodies, which may be aggregates of hyphae, or stout-walled sacs containing spores. In one large specimen, the masses at the base consisted of hyphae only and were small, up to  $0.5 \times 0.4$  mm. in cross-section; above these were similarly shaped bodies, up to  $1.2 \times 0.6$  mm., but with a stout sub-translucent wall, open above, and filled with spores. More usually, in open specimens, the basal part consists of closed sacs containing spores, while the upper part is formed of similar sacs open above. These sacs are loosely united to one another by hyphae, and the interspaces contain fragments of the bark tissue of the host. In the upper part of the cup the spores and the gelatinised remains of the walls of the sacs are fused into a continuous mass, while the fragments of bark form the brown reticulated network on the surface and thus mark the boundaries of the separate sacs.

The specimens from which the genera *Artocreas* and *Matula* were described are full-grown and open. Unopened examples are globose (Plate III, figs. 2 and 3), enclosed in a stout wall, except at the base. In these specimens, the sacs are, in general, in two layers, with a distinct zone of separation, partly filled by loose hyphae, in the equatorial plane. The sacs in the lower half arise from the host tissue at the base, those in the upper half are united to the upper part of the wall of the fungus. In general, all the sacs contain spores. In a horizontal cross-section the shape of the sacs is irregular (Plate III, fig. 5); their outline is variously lobed, and the lobes of adjacent sacs interlock without any definite arrangement. The fungus consists of a group of sacs, enclosed in a stout universal wall, with which the outer walls of the peripheral sacs are fused, while their inner walls are only loosely united to one another.

Dehiscence is first indicated by a depression at the apex of the sphere. Sections of such an example (Plate III, fig. 4) show that there is already a cavity in the upper part of the fungus, due to the gelatinisation of the walls of the uppermost sacs. The upper part of the wall then gelatinises, and the fructification expands, becoming widely open and cup-shaped, the gelatinised walls of the upper sacs and their spores forming a continuous mass which is hard and compact when dry. In this stage, parts of the walls of the sacs persist in the upper region, and closed sacs are present in the lower, more or less as figured by Masee. Subsequently the walls of the lower sacs are gelatinised, and all the spores disappear, leaving the empty cup.

Masee's generic description is obviously incorrect. The fungus is closed at first, but it is not closed by an epiphragm. He did not have any closed specimens. Further, the internal

tissue is not a gleba broken up into numerous loculi by dissepiments, but a group of sacs attached either to the host tissue at the base or to the wall, and loosely bound together elsewhere by hyphae.

The latter feature of the fungus is most remarkable. In a sense, the growth of the lower group of sacs is independent of the universal wall which later forms the cup. The fungus arises within the cortex of the host plant and forms a small pulvinate cushion which ruptures the epidermal layer. The hyphae of this cushion do not arise from the host tissue continuously over the whole of its base, but from a circular peripheral zone and from separate small areas enclosed by that zone. This is also evident when tangential sections of the host are taken over the area of attachment of the mature cup. One then finds that the sections consist of cortical tissue in which are embedded isolated groups of spores. Thus, the bases of the lower sacs are embedded separately in the cortex. It is therefore clear that the lower sacs arise from separate points of the host tissue distinct from one another and from the universal wall. Hence the hyphae from which these sacs are formed must arise from the host tissue in separate masses. The outer layer, or universal wall, of the fructification arises from a circular zone surrounding the points of origin of the sacs.

The fusion, or partial fusion, of these masses occurs before the fungus emerges from the cortex. In all cases the emerging pulvinate cushion presents a continuous surface. In small examples, there may be only one layer of sacs; the outer walls of the peripheral sacs are then fused with the universal wall, as are also the apical parts of the walls of the inner sacs. Interspaces, filled with loosely interlacing hyphae, are found, in such examples, only vertically between the sacs. In these cases, it would appear that the hyphae of the universal wall form a tissue continuous with the upper parts of the sacs during the whole period of development of the fungus.

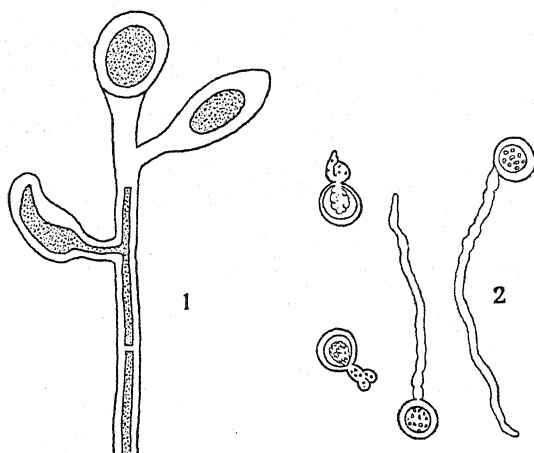
In larger examples, however, the sacs are situated more or less in two layers, the lower arising from the host tissue at the base of the fungus, and the upper united to the universal wall, with interspaces between the two layers. In these examples, it would seem that the sacs first formed are attached to the upper part of the wall and are succeeded by others from the host tissue within the base as the fungus increases in size.

The independent initiation of the sacs at the base of the fungus explains the presence of bark fragments in the mature fructification. As each group of hyphae emerges from the cortex, it pushes up pieces of the overlying tissues, and these are retained between the sacs. The fact that these fragments are found

between the two layers of sacs in the larger specimens favours the view that in such examples the basal sacs succeed the upper.

In some slides, however, the vertical section of an unexpanded specimen clearly shows a small immature sac arising from the upper part of the universal wall and pushing between two mature sacs. Hence, the sacs can arise from the wall as well as from the host tissue at the base.

Frequently the fungus does not form a cup. Minute pulvinate examples, about 1 mm. diameter, which barely emerge from the ruptured cortex, may contain mature spores. In these the upper layer disappears, leaving a shallow cavity filled with spores.



Text-fig. 2. *Arlocreas poroniaeforme*. 1. Conidiophore.  $\times 600$ . 2. Germinating conidia.  $\times 250$ .

The sacs are completely filled by the conidia and conidiophores. The conidiophores arise from the wall of the sac, and are hyphae of varying length which bear conidia terminally and laterally. The apical conidium is the first to be formed; consequently the mature spores are found at first in the centre of the sac and the immature spores towards the periphery of the spore mass. The conidiophores and conidia adhere strongly to one another and form a solid mass, so that it is practically impossible to tease out or separate the contents of an unopened sac in order to ascertain the mode of production of the conidia. But in open sacs, where gelatinisation and deliquescence of the older conidiophores has begun, immature conidiophores may be isolated. Paraphyses have not been observed.

The conidiophores are  $4-6\mu$  diameter, with a thick wall, and



a narrow lumen,  $1-2\mu$  diameter. The immature conidium, on staining, appears obclavate or obpyriform, but this is only the shape of the cell contents. The wall of the spore is thick, and usually fused with adjacent structures, so that its outline is not evident. The general shape of the immature spore is elongated oval, often irregularly oval, the thickness of the wall varying from point to point.

The terminal spore is produced first. Others are subsequently produced basipetally on short lateral branches. Sometimes these arise close below the terminal spore, so that a cluster of conidia results. The cell contents increase in size and form a spherical mass situated towards the base of the spore, and this mass then acquires a thick wall independent of the wall of the parent cell. The spores are liberated by the deliquescence of the wall of the parent cell. At first, shreds of the latter may remain attached to them, and their outline appears irregular, but they subsequently become quite smooth.

The mature spores are hyaline, spherical,  $16-20\mu$  diameter, with a wall  $1.5-2.5\mu$  thick. In some sections of the fungus, stained with Haematoxylin or Bismarck Brown, a few of the spores are black; these latter have granular contents which appear to be disorganised. With Gentian Violet, the immature spores are strongly stained, but the mature spores do not take the stain.

In the immature fungus, the internal tissue is dull yellow and subgelatinous. When the spores are mature, it becomes opaque.

In its conidiophores and the mode of formation of the conidia, *Artocreas poroniaeforme* resembles *Michenera artocreas*. In both the conidia are produced basipetally, and are formed within a spore mother cell. In the latter species, the wall of the mother cell persists as an apical appendage and a basal pedicel, but in *Artocreas poroniaeformis* this wall gelatinises and disappears, leaving the spore spherical.

On the other hand, the general structure and mode of development of *Artocreas poroniaeforme* is quite different from that of *Michenera artocreas*, and the two cannot be regarded as co-generic. In that respect, Masee's conclusion was correct, but it would seem doubtful whether he was justified in rejecting Berkeley and Broome's generic name, *Artocreas*.

Both Pierce and Patouillard suggested that the spore of *Michenera* was a chlamydospore. This question was discussed at length by Lyman, who noted that the formation of the spore was similar to that of a chlamydospore in that the protoplasm shrank into the central portion of the cell, leaving empty walls above and below.

Lyman pointed out two distinctions between the *Michenera*

spores and ordinary chlamydospores. In the first place, the *Michenera* spore is normally formed in a terminal cell, not in any cell, and the empty portions of the parent cell are differentiated into a stalk and an appendage which persist as permanent parts of the spore. But he noted that in some *Michenera* spores the appendage was absent, while in other cases it grew out as a hypha, "perhaps" with the formation of other spores beyond, so that the spore was intercalary like an ordinary chlamydospore.

The second distinction is that the *Michenera* spores are grouped in definite fructifications. In this connection Lyman stated that "No such condition is known in the case of undoubted chlamydospores, but the nearest approach is found in *Nyctalis*, *Ptychogaster*, and *Fistulina*, where extensive chlamydosporic fruit bodies are formed, though there is no definite hymenium and the fructifications are formed through the usurpation by the chlamydospores of what were primarily basidiosporic fructifications."

Lyman concluded that it seemed impossible to avoid classing the spores of *Michenera* as chlamydospores, since their method of formation is chlamydosporic.

The method of spore formation in *Artocreas poroniaeforme* is the same as that in *Michenera artocreas*, but no part of the wall of the parent spore persists. Intercalary spores have not been observed in *Artocreas poroniaeforme*. The constant character of the conidiophore and conidia would appear to distinguish these spores at least from chlamydospores, as usually understood. If they are to be classed as chlamydospores, then all "endospores" must be similarly regarded; and though the mode of formation may be similar, there does not appear to be anything to be gained by thus extending the usual conception of a chlamydospore.

#### MATULA ROMPELII RICK.

Specimens of *Matula Rompelii*, which was described by Rick from Brazil, have been kindly furnished by Mr C. G. Lloyd. In general appearance the open fungus is identical with *Artocreas poroniaeforme*, though slightly different in colour. It was described as yellow. The cups, in the available specimen, are up to 4 mm. diameter and 2.5 mm. high, minutely tomentose externally. The spores are spherical, 16-19 $\mu$  diameter, with a wall up to 3 $\mu$  thick. The specimens are in an advanced stage, but they show the remains of the walls of the sacs at the base, and in one a closed sac, filled with spores, was found.

The spore mass is more friable than in the Ceylon species, but, nevertheless, the spores are held together by the disorganised

remains of the conidiophores, etc., when the mass is sectioned. The wall of the cup arises in a ring, as in the Ceylon species, but there is, in the specimens examined, a thin continuous layer, about  $12\mu$  thick, overlying the host tissue and forming a basal layer within the cup. A few cortical cells of the host plant occur in the tissue of the lateral wall of the cup near the base, but these do not appear to occur between the sacs. This difference from the Ceylon species may be due to a difference in the host plant, or, more probably, to the presence of a basal layer of hyphae within the cup.

*Matula Rompelii* is co-generic with *Artocreas poroniaeforme*, but it is doubtful whether it is identical. The two differ in structure as regards the base of the fungus, though this difference may not be constant. But both are conidial forms, and it is probable that they are conidial forms of different species.

*PENIOPHORA HABGALLAE* (B. AND BR.) COOKE.

*Peniophora Habgallae* Cke. (Plate II, fig. 2) also occurs on fallen Camphor twigs at Hakgala. This species is white, sub-translucent, saucer-shaped or pezizoid, circular or oval, regular or slightly lobed, up to 1.5 cm. diameter. The upper surface is plane or undulating, with a sharp definite margin, from which the convex sides curve backwards slightly into the flat base, which is erumpent from the host plant only over a central area. In some examples the base is contracted into a short broad stalk. When fresh, the fungus is up to 3 mm. thick, but in drying it shrinks into a thin disc, 0.5–1 mm. thick, the lower surface then becoming concentrically grooved. The hymenial layer is white, or pallid with a white margin, fleshy, about 0.15 mm. thick, and the cortex at the sides is white, rather loosely built, about 0.1 mm. thick. The internal tissue is subgelatinous, and the fungus is fragile when fresh. The cystidia are hyaline, flask-shaped or conical, encrusted with a slightly thickened wall, 80–100 $\mu$  high, 14–20 $\mu$  diameter below. The spores are oval, smooth, hyaline, 16–21  $\times$  12–14 $\mu$ .

*Peniophora Habgallae* occurs on the lower side of the Camphor twigs, and *Artocreas poroniaeforme* on the upper side.

At first sight, these two species do not appear to have any relationship to one another. But on cutting vertical sections of the *Peniophora*, especially of the examples which arise from a pseudo-stalk, it is found that the structure of the base of the *Peniophora* is exactly the same as that of the *Artocreas*. The internal tissue at the base of the *Peniophora* consists of ovoid masses, loosely united by hyphae, with fragments of the cortical tissue of the host between them (Plate III, fig. 6). They are identical with the sacs of *Artocreas*, but, so far as has been

observed, they do not contain any spherical conidia. The walls of the sacs are interrupted above, and the hyphae grown out and intermingle to form the continuous context of the *Peniophora*. This structure is not universal; in some examples the fungus grows out directly from the host without forming separate masses of hyphae internally.

In the case of *Corticium subgiganteum*, examples occur in which the *Corticium* and the *Michenera* fructifications are combined. I have never found that state in *Peniophora Habgallae*. The *Peniophora* and the *Artocreas* fructifications are, so far as has been observed, always separate.

#### CULTURES.

When Camphor twigs bearing *Peniophora Habgallae* only were placed in a closed glass dish with the *Peniophora* uppermost and kept moist, white globose bodies, up to 3 mm. diameter, developed on the hymenium of the *Peniophora*. These were composed of uniform hyphal tissue internally, and they became covered by a hymenial layer which contained typical *Peniophora* cystidia and immature basidia (?). Spores were not produced. The same occurred when other twigs were placed so that the *Peniophora* was on the under side.

Twigs bearing *Artocreas poroniaeforme* only, when similarly treated, produced similar globose *Peniophora* stromata on both the upper and the lower side, independently of the position of the *Artocreas*. The *Peniophora* stromata developed both on the bare side of the twig and among the *Artocreas* fructifications. But no growth occurred from the *Artocreas*.

Specimens of *Peniophora Habgallae* were collected at the beginning of August 1924. It was not found possible to obtain spore deposits from these, and consequently tissue cultures were instituted on maize meal agar. These developed a white fleecy mycelium. Transfers of mycelium were made to tubes of maize meal agar and to sterilised Camphor twigs in Roux tubes. The twigs were cut from living branches, sterilised by boiling three times and then autoclaved in the tubes.

On maize meal agar slants, the mycelium developed white globose bodies, up to 3 mm. in diameter. These were of uniform structure internally. They did not bear cystidia or basidia, or any form of spore.

In the Roux tubes, the mycelium grew superficially over the Camphor twig, covering it with a white film, and globose bodies, similar in appearance to those on maize meal agar slants, were produced on this mycelium, chiefly at the upper end of the twig. These cultures, which were begun on September 14th, were not examined until December 21st, and they had dried out in the

meantime. On examination it was found that these were identical in structure with immature *Artocreas*, and some of the sacs contained spores.

In these culture specimens, the universal wall is thin and not sclerotoid. The internal tissue is dull yellow and subtranslucent in section, and traversed by white veins which consist of the loose hyphae between the sacs. In immature specimens of *Artocreas poroniaeforme*, naturally developed, these veins appear brown, because of the inclusion of fragments of bark, but no bark fragments were found in the culture specimens, no doubt because these developed from superficial mycelium, not from within the cortex. Numerous spore mother cells were present in some of the sacs, but few spores; and the latter were, in general, thin-walled, only one or two having a wall up to  $1.5\mu$  thick. Some of the spores were much larger than in nature, and attained a diameter of  $32\mu$ .

The typical cup-shaped fructification was not obtained. Similarly, Lyman did not obtain the cup of *Michenera artocreas* in his cultures. Under the conditions of the cultures, the walls of the hyphae were not thickened, nor, in general, was the wall of the conidium. But there can be no doubt, from the structure of the fructification and the mode of formation of the spores, that these examples are *Artocreas poroniaeforme*.

Recently developed specimens of *Artocreas poroniaeforme* were collected in December 1924, and spores suspended in hanging drops of water, maize meal agar, Raulin agar, and Naegeli agar in damp cells. Germination was observed only in drops of the two last-named, which had been allowed to dry out in twenty-four hours and then re-wetted; these spores germinated two and a half days later. Similar treatment of the water and maize meal agar drops did not induce germination. The germ tube emerges through a minute perforation in the thick wall and at first is oval or irregularly lobed. Subsequently it develops into a stout hypha, about  $4.5\mu$  diameter. Transfers of these germinated spores to maize meal agar were unsuccessful owing to contaminations.

#### CONCLUSIONS.

The cultures prove that *Artocreas poroniaeforme* is a conidial form of *Peniophora Habgallae*, as was suspected from the association of the two fungi and the similarity of the structure of the base of the *Peniophora*, in some examples, to that of the *Artocreas*. *Artocreas poroniaeforme* consequently affords a parallel to *Michenera artocreas*, which has been shown by Lyman to be a conidial form of *Corticium subgiganteum*.

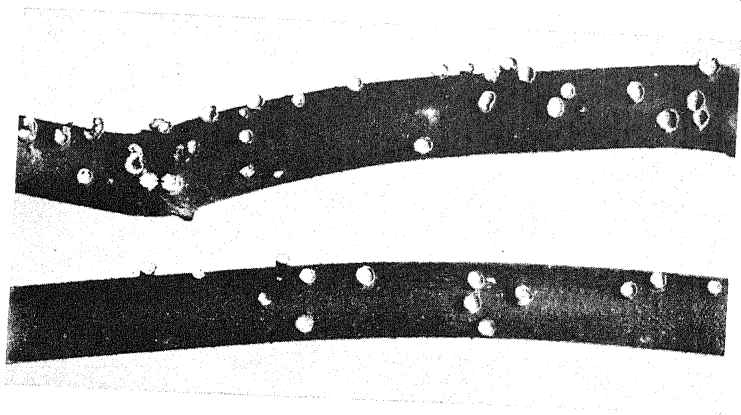


Fig. 1.

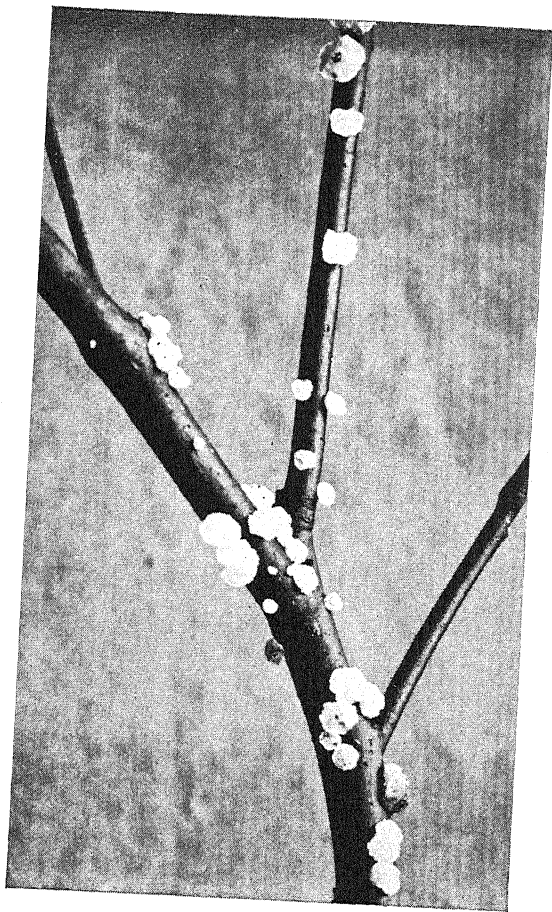
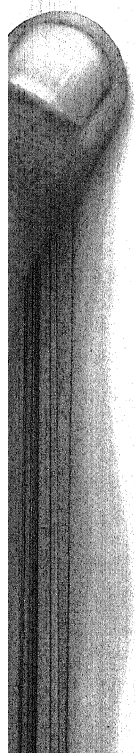
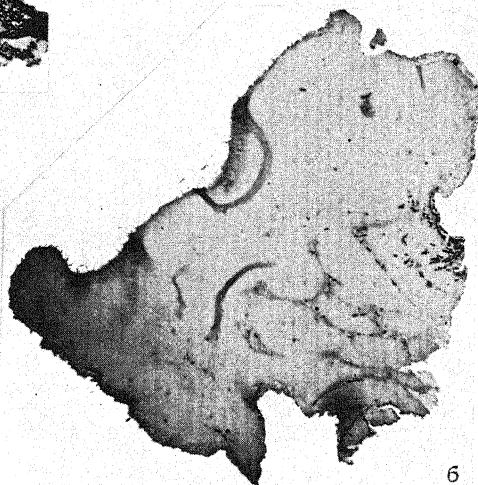
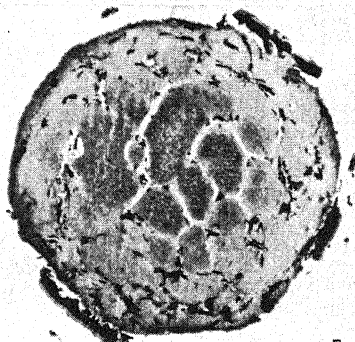
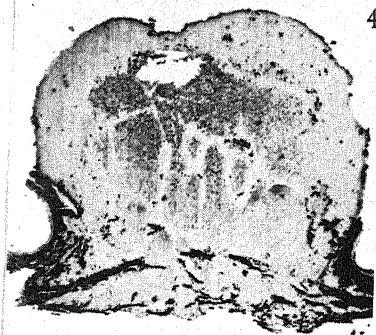
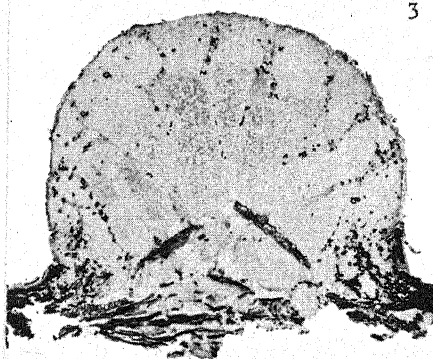
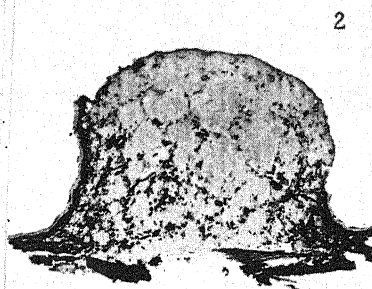
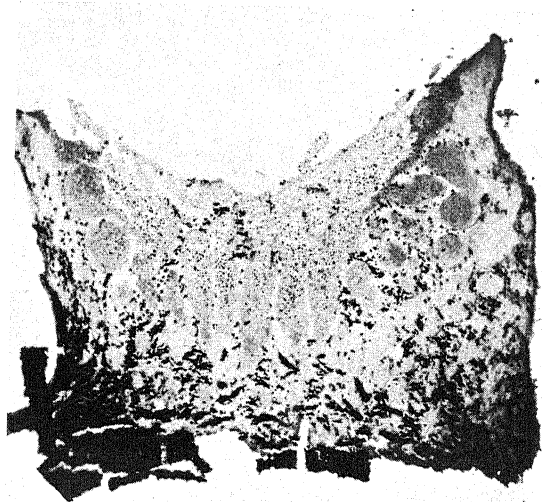


Fig. 2.









The mode of formation of the conidia in *Artocreas* and *Michenera* is the same. In each, the conidium is formed in a spore mother cell, and the successive spore mother cells are produced basipetally on the conidiophore. But in *Michenera* the wall of the mother cell persists and forms an appendage, while in *Artocreas* it gelatinises and disappears.

The structure of the fructification of *Artocreas* is, however, quite different from that of *Michenera*, and the two genera cannot be combined.

It would appear that Berkeley and Broome's name *Artocreas* should stand, its withdrawal having been due to the mistaken supposition that *Artocreas* was identical with *Michenera*.

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## EXPLANATION OF PLATES II AND III.

## PLATE II.

- Fig. 1. *Artocreas poroniaeforme*.  $\times \frac{1}{2}$ .  
Fig. 2. *Peniophora Habgallae*.  $\times \frac{1}{2}$ .

## PLATE III.

- Fig. 1. Vertical section through an open specimen of *Artocreas poroniaeforme*.  $\times 12$ . The dark irregular bodies are fragments of bark.  
Fig. 2. Vertical section through a small immature unopened specimen.  $\times 15$ .  
Fig. 3. Vertical section through an unopened specimen, nearly mature.  $\times 15$ .  
Fig. 4. Vertical section through a specimen just about to expand.  $\times 15$ .  
Fig. 5. Horizontal section through an unopened specimen.  $\times 20$ .  
Fig. 6. Vertical section through a stroma of *Peniophora Habgallae*.  $\times 15$ . The two thick curved bands are due to folds in the section.

## THE TOXICITY OF THE SPORES OF *TILLETIA TRITICI* TO ANIMALS\*.

By Norman Dobson, B.Sc., M.R.C.V.S.

THE possible toxicity of grain damaged by bunt or smut (*i.e.* infected with the spores of *Tilletia Tritici* or *Ustilago* sp.) when fed to animals has been the subject of considerable controversy and references in the literature which have a bearing on the point are peculiarly contradictory. The question, apart from its scientific interest, is one of considerable economic importance. In view of the fact that Professor J. Russell Greig had observed an apparent connection between the presence of the spores in the tissues of dogs and the occurrence of epileptiform convulsions in these animals<sup>(4)</sup> it was considered desirable that feeding experiments under strictly test conditions should be instituted. An application was therefore made by Dr Malcolm Wilson and Professor Greig to the Board of Agriculture for Scotland for a grant in aid of the expenses of such research, and in June, 1924, I was entrusted with the prosecution of the work under their supervision.

By the kindness of Professor W. Wright Smith and Principal O. Charnock Bradley, adequate facilities were provided for the investigation at the Royal Botanic Garden and at the Royal (Dick) Veterinary College, Edinburgh, respectively.

Tubeuf<sup>(11)</sup> states (p. 306) that certain diseases are produced in animals by the consumption of spores of smut fungi with food and *Tilletia Tritici* is considered as one of the chief causes of these troubles. "The symptoms in the few cases of disease observed do not agree very closely. A paralyzing effect on the centres of deglutition and the spinal cord seems to be regularly present. As a result one generally finds a continuous chewing movement of the jaws, and a flow of saliva, also lameness, staggering, and falling. Cattle, sheep, swine, and horses are all liable to attack."

Liskum and Krastavizky<sup>(7)</sup> fed up to 10 grams of spores of *Tilletia Tritici*, and *Ustilago Maydis*, daily to guinea-pigs, mice, rabbits, and a dog. During the experiment no injurious effects were seen, and all the subjects developed more or less normally. *Post mortem* examinations, however, showed that all their organs were attacked by the spores, and partially destroyed. The typical symptoms noted were, hyperaemia of the digestive tract, dark or grey coloration of the mucous membrane of the intestines, and stomach, and hyperaemia of lungs, kidney and

\* I am indebted to the Board of Agriculture for Scotland for a grant to defray the expenses of this research.

brain. The spores were found to have accumulated especially in the adrenal bodies and the blood vessels were so full of the spores, that they sometimes burst from obstruction. In one case, the writers observed that they had penetrated the placenta, and reached the tissues of the foetus. It was noted that there was almost always a disproportion between the number of spores and the pathological changes. The conclusion arrived at was that the spores of *Tilletia Tritici* and *Ustilago Maydis* are injurious to animal organs, though their effects may not always be visible externally.

Lindau in Sorauer<sup>(9)</sup> doubts the toxic effects of the spores of *Tilletia Tritici*, as he quotes the records of Staes, who noted that a farmer regularly fed his horse and cattle with waste meal, in which a very large quantity of the spores was present. The meal was always soaked in water twenty-four hours before being used for feeding, and as this probably caused most of the spores to germinate they would be killed by passage through the intestines. Lindau evidently considers this a proof that the spores of *Tilletia Tritici* are not injurious to animal tissues. It would appear that he was mistaken in assuming that such immersion was sufficient for germination as Riehm<sup>(8)</sup> found that three or four days in diffused light at a temperature of 20° C. was required: direct sunlight or total darkness delayed germination, as also did temperatures below 20° C. In the present experiments it was found that in summer time under diffused light, at the ordinary temperature of the laboratory, six or seven days were required for germination.

It would seem that if spores were fed to animals in a germinated condition, there would be a greater possibility of any toxic substance being absorbed by the animal tissues, owing to the rupture of the hard spore case. When examining the faeces of the experimental animals, it was extremely rare to find any of the spores ruptured. Only in the specimens from the guinea-pig and the rabbits were broken spores found, and presumably this occurred during the process of mastication, due to their fine molar teeth. In no case was there any sign of germination of the spores found in the faeces.

Baudys<sup>(1,2)</sup> gives a graphic account of his experiments upon white mice, fowls, rabbits, and himself, although he does not state definitely the weights of the spores administered to the animals or to himself. In view of negative *post mortem* findings, and the fact that he did not experience any discomfort from taking a feed of 9.5 grams of the smutted wheat mixed with wheat meal, he concluded that the spores are not poisonous. Klimmer<sup>(6, p. 138)</sup> refers to the work of Albrecht, "in a case where fifty-one head of cattle fed on wheat chaff infected with

*Tilletia Caries*, *Puccinia graminis*, and *Pleospora herbarum*, eight beasts were taken ill, and three died." The symptoms were—spasms of the muscles of mastication, weakness with anaesthesia in the lumbar region, and fever; inflammation of the conjunctiva, mucous membrane of the nose, and upper air passages; urgent desire to urinate, and tenesmus; on *post mortem*, gastroenteritis with erosion was found.

Greig(4), in 1922, published a report of three cases which he had attended of epileptiform convulsions in the dog, which appeared to be associated with the spores of *Tilletia Tritici*. "The symptoms were identical in each case. The animal was suddenly attacked by a series of convulsions, which continued for some hours, and terminated in coma; in two cases death resulted, but in the other case recovery took place, though the animal was subsequently destroyed."

In case A the faeces contained a very large number of spores of *Tilletia Tritici*. An examination of the abdominal organs was made *post mortem*, but no definite abnormality was found.

"In case B the faeces contained large numbers of the spores, which were found on *post mortem* to be present throughout the alimentary tract; they were also very numerous in the bile and urine, which in this case were examined microscopically. The brain unfortunately was not examined, and no definite microscopic lesion was discovered in any other organ.

"In case C, spores were present in large numbers in the intestine and urine. No definite lesion was remarked until the cranium was opened, when an acute cerebral meningitis was observed; smear preparations from the cerebral cortex again revealed the presence of the spores. In sections cut from the brain, kidney, liver and spleen the blood vessels were seen to contain very large numbers of dark pigmented bodies; these showed much variation in size, ranging from mere particles of pigment, to bodies  $12\mu$  in diameter; their nature is yet to be determined. Since the spores were so numerous in the urine, it was expected that they might also be present in the blood stream. No spores have, however, been seen in sections of tissue containing blood vessels, and it is possible that these pigmented bodies may represent spores in a state of disintegration; on the other hand, they may be mere artefacts."

Greig pointed out that the spores of *Tilletia Tritici* were frequently present in the faeces of normal dogs, but in many cases lengthy search was required before a single spore could be demonstrated, whereas in the faeces of affected cases, commonly six and not infrequently twelve spores could be seen in a single field. He suggested that although his material was insufficient to permit of any definite conclusion, it appeared

probable that on occasion the spores of *Tilletia Triticum* might constitute a factor in the production of epileptiform convulsions.

The preliminary work of the present investigation consisted in the examination of normal dogs with the object of determining the incidence of the spores of *Tilletia Triticum* in the alimentary tract. Three hundred and fifteen patients at the Royal (Dick) Veterinary College, were examined, and among these there was, of course, a wide diversity of breed. A large percentage were house dogs of the city, but there were also a large number of sporting dogs of various breeds.

#### METHODS OF COLLECTION.

The method of collection, chiefly consisted in direct removal of the faeces from the rectum. In some cases, where faeces had recently been passed in the kennel, a sample was taken from the centre of the stool, transferred to a sterile Petri dish, and emulsified in normal saline solution. With a glass rod, dropping the same quantity constantly, some of the emulsion was transferred to a microscopic slide, and covered with a  $\frac{3}{8}$  in. square cover slip. The entire  $\frac{3}{8}$  in. area was examined and a record kept of the number of the spores found. Two such preparations were made at each examination of faeces. During the early part of the experiment spores were found only in small quantities in the faeces, whereas towards the close of the feeding period the  $\frac{3}{8}$  in. area showed the presence of seven to eight hundred spores in almost every examination.

The dog was examined as early as possible after its arrival at the hospital, again on the second day after admission, and subsequently according to the length of time it was a patient in the hospital. Only in two cases were spores of *Tilletia Triticum* found in the faeces of new arrivals, whereas when the dogs had been in hospital two days, it was found that 26.6 per cent. were passing the spores in their faeces.

The bedding of the canine patients in the College consists of wheat straw and sawdust. Systematically on arrival of the wheat straw at the hospital from the forage merchant, washings were made and centrifuged. Examination of these microscopically showed the spores very often present, especially in the months of July and December, 1924, and during these months, examination of the faeces from the patients, showed heavy infestation. During the months of June, August, September, October, and November the washings of wheat straw showed a less gross infestation, and the examination of the faeces showed a similar decrease.

Periodic microscopic examinations were made of the food-stuffs given to the dogs. The biscuits seemed a likely source

of infection, but spores were not found in these. Washings of the coats of the dogs gave positive results, and large numbers of spores were found during the months of heavy infection of the bedding.

One therefore must assume, that in these cases arriving at the hospital quite clear of infection and which in two days were found to be passing spores in their faeces, infection occurred through wheat straw contamination of food placed in a shallow bowl on the floor of the well-bedded kennel.

#### EXPERIMENTAL ANIMALS: HOUSING AND FEEDING ARRANGEMENTS.

Of three rooms at my disposal, in addition to the research laboratory, one room was utilised as a food preparation room, and the remaining two for the accommodation of the experimental animals. These were all admirable rooms, with concrete floors, washable walls, well lighted and ventilated. The experimental animals were two dogs, eight rabbits, eight guinea-pigs and, later, four fowls. The dogs were accommodated with benches well supplied with sawdust, and had the use of one room. The rabbits, guinea-pigs and fowls were kept in individual cages fitted with zinc trays, and a thick bedding of sawdust. The cages were placed on benches and one side of the cage was fitted with a zinc plate preventing the passage of food, etc., from one cage to the next. The dogs, rabbits and guinea-pigs were kept three weeks before the special feeding commenced, and the examination of the faeces during the whole period showed every animal to be quite free from infection with the spores of *Tilletia Triticis*.

#### CONTROLS.

One rabbit and one guinea-pig were used as controls. They were kept under precisely similar conditions to those of the other animals, with the one exception, that they received no spores.

From the date of arrival of the animals until May 3rd, 1925 (a period of nineteen weeks), when the fowls had to be placed in the same room as the rabbits and the guinea-pigs, the faeces of the controls were quite free from spores. Periodical sweepings were taken from the ledges and window-sills and examined for spores but always with negative results until the arrival of the fowls, and then, presumably as a result of their constant scratching in the cages, spores were found very occasionally in the faeces of the controls and in the sweepings of the ledges, etc.

This condition might be contrasted with that prevailing in the workroom where the experimental animals were kept by

Baudys(2). He describes the atmosphere as saturated with the spores, causing irritation to mucous membrane of nostril and sneezing.

#### FEEDING ARRANGEMENTS.

The food was prepared in the room specially set apart for the purpose. The ears of wheat were previously examined and bunted grain detached. The diet of the dogs consisted of unbroken biscuit in the early morning and at four o'clock in the afternoon they were given broken biscuits over which had been poured the liquid from boiled meat with a little minced meat added. This gave a moist mixture and with the aid of forceps the bunted grains were introduced under the surface of the food in the bowl and crushed, releasing the spores from the grain without contaminating the atmosphere. The rabbits and guinea-pigs were given a few oats with greens and roots in the early morning; the afternoon feed consisted of moistened oats with the spores added as detailed in the case of the dogs.

#### *Tabulated Results of Experiments.*

1. Number of days the spores were fed to the animals.
2. Total quantity of spores fed (in grams).
3. Weight (in grams) of animals when special feeding began.
4. Gain or loss in weight (in grams) during the period.

Animal	1	2	3	4
Dogs.				Gain
DA	136	100.62	} No facilities for weighing	
DB	137	103.57		
Rabbits.				
RA	14	.375	1558.6	259.8
RB	142	54.00	1488.7	941.3
RC	49	3.50	1671.8	147.2
RD	107	15.007	1507.0	798.0
RE	120	21.32	1464.4	719.4
RF	149	85.00	1388.8	975.2
RG	172	Control	1067.5	890.0
RH	28	1.25	1499.5	108.8
Guinea-pigs.				
PA	14	.375	435.2	40.4
PB	162	Control	657.6	316.0
PC	119	20.60	500.8	180.0
PD	149	85.00	341.6	302.01
PE	50	3.64	443.5	156.50
PF	137	40.43	586.7	105.69
PG	28	1.03	565.7	41.3
PH	106	14.7	559.7	164.59
Fowls.				Loss
FA	31	36.82	1040.54	52.54
FB	52	207.00	1360.18	96.75
FC	52	207.00	1476.13	48.67
FD	19	11.80	1101.22	259.21—Roup case.



## CONDITION OF THE ANIMALS DURING THE EXPERIMENT.

The health of the dogs, rabbits and the guinea-pigs was excellent throughout the period. The animals all gained weight and no abnormal symptoms were seen. All remained keen for food until the dosage reached 5 grams daily and then occasionally some inappetence was shown which could be attributed undoubtedly to the obnoxious, but very characteristic odour, of the *Tilletia Tritici* spores, the "herring brine" odour due to the presence of trimethylamin.

Four fowls were purchased on May 2nd, 1925, and as the faeces showed no sign of spores the special feeding commenced at once. Two of the fowls were fed with 10 grams of the spores of *Tilletia Tritici* per day for the ultimate fourteen days of the feeding experiment and showed no pathological symptoms. Fowl FD was killed on May 23rd owing to the occurrence of roup which developed on May 16th, after nineteen days special feeding: it showed the characteristic lesions on the comb, wattles and mucous membrane at the commissures of the mouth.

It is interesting to note that according to Heald<sup>(5)</sup> each smut ball contains from six to nine million spores. From experience it was found that the average of fifty-six smut balls to the gram was common and therefore the mass infection with the spores can be seen at a glance from the above table.

## POST MORTEM FINDINGS.

The *post mortem* examinations revealed healthy organs in every case. In the animals that had been subjected to feeding for a month and more fat was found in the usual fat deposits in large quantities. Urinary and gall bladders, after ligature, were removed to sterile Petri dishes and here incised and the contents collected. Examinations of the bile and urine for spores in all cases proved negative. The stomach and intestines were quite normal, there were no petechiae, haemorrhagic patches or discoloration on the surface of the lungs or on any other serous membranes, and the kidneys, adrenals, spleen and brain were all normal. The blood was carefully examined, both from the heart and from the peripheral vessels, and found to be free from spores and no sign of rupture of the blood vessels was detected. Portions of the various organs were removed and fixed in Flemming's weak solution or in Pick's solution, passed through the usual processes, embedded in paraffin, and serial sections made and stained with Mayer's haem-alum. Examination of these sections proved entirely negative; the spores of *Tilletia Tritici* were not found in any cells, blood vessels, or

embedded between tissue cells, in any organs, neither was any cell destruction found.

The only two animals that became pregnant were guinea-pig PH and rabbit RF. Guinea-pig PH aborted on February 1st, 1925, after being under test since December 22nd, 1924, and must have been mated prior to this date. Rabbit RF was mated on May 1st, 1925, and destroyed June 11th, 1925, when five full-term young were found. In neither of these cases could the spores of *Tilletia Tritici* be found in the foetal membranes or in the organs of the foetuses. In all the fowls large deposits of fat were found and the liver in the cases FB and FC was slightly fatty, otherwise all the organs appeared quite normal. The tissues of the fowls were examined microscopically with negative results and in none of the organs was cell destruction visible.

From the foregoing and the table giving the increase in weight of the different animals, it will be seen that the non-appearance of pathological symptoms is quite compatible with the perfectly healthy condition of all the animals during the experiment, their general appearance of healthy vigorous condition in life and negative *post mortem* results.

The loss of weight in the fowls may be accounted for by the fact that they had previously been kept in a large run permitting all necessary exercise and, when confined in a large cage, with great lack of exercise and little scratching accommodation, although the food was suitable, loss in weight resulted but the condition of the organs remained healthy. The loss in weight is not to be attributed to any toxic effect of the spores, but rather to the fact that the condition of these birds suffered from their confinement. One must also remember that, for a period of fourteen days, they were consuming ten grams of the spores per day; these were devoured eagerly, although their food value was practically nil.

The question arises whether the spores which have passed through the alimentary tract are again capable of causing infection. Tubeuf<sup>(12)</sup> has shown that the passage through the alimentary tract destroys the germinating power of the spore and therefore manure contaminated with spores when spread on the land does not infect the crop. Baudys<sup>(1)</sup> also came to the same conclusion. Steglich<sup>(10)</sup> considers that danger from spores introduced with stall manure lasts but a few days because, as he asserts, manure acts as a stimulant to germination and thus causes the spores quickly to become innocuous in the absence of a host plant. Dreger<sup>(3)</sup> states that grain infection is perpetuated in the manure. Several experiments were made with the spores that had passed through the alimentary tracts of

the various test animals during the investigation, but germination could not be induced.

#### DISCUSSION AND CONCLUSIONS.

Bunt of wheat has been known from very early times and is referred to by Theophrastus and other early Greek and Roman writers. Until 1775 it was believed to be caused by environmental or providential influences, but in that year Tillet proved its infective character by experiment. Much research has been carried out on the biology of this fungus and the subject still occupies a prominent position in economic botany. Under these circumstances, if the feeding of the spores to animals had commonly proved injurious, one would expect that more precise records of its toxicity would have been made. The early records of Tubeuf<sup>(11)</sup> and the findings of Liskum and Krastavizky<sup>(7)</sup> and Greig<sup>(4)</sup>, who either found that the spores had penetrated the tissues or noted pathological symptoms associated with the occurrence in feeding stuffs, still remain. A possible explanation of their observations may be found in the assumption that such effects as they noted resulted from the occasional presence of an unknown factor which allows of the absorption and penetration of the spores.

The result of this investigation, however, taking into account the lengthy period of the test, the large quantity of the spores administered, the invariable health of the animals throughout the entire experiment and the negative *post mortem* findings, justifies the conclusion that only in the most exceptional cases can the spores of *Tilletia Tritici* be regarded as injurious to animals. This is in agreement with the later work of Tubeuf<sup>(12)</sup>. From the point of view of the practical stock feeder, it may be safely accepted that grain infected with bunt may be fed without injury to animals.

#### ACKNOWLEDGMENTS.

My special thanks are due to Dr Malcolm Wilson and Professor J. Russell Greig for their unfailing kindness to me whilst the experiments were in progress, and for their assistance in the preparation of this paper. I should like also to express my gratitude to Professor E. S. Salmon, of the South-Eastern Agricultural College, Wye, who, through the instrumentality of Dr Malcolm Wilson, kindly supplied all the bunted ears of wheat for this experimental work.

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## PSEUDOPERONOSPORA HUMULI AND ITS MYCELIAL INVASION OF THE HOST PLANT.

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(With 4 Text-figs.)

THE Downy Mildew of the hop, *Pseudoperonospora Humuli* (Miy. and Takah.) Wils., first found in Europe in 1920(6) in the Experimental Hop-garden and Nursery at Wye College, Kent, is now so widespread in this country as to appear indigenous. During the subsequent years it has appeared to an increasing extent on wild hops and in cultivated hop-gardens generally, and has shown during 1925 that it is a menace to be seriously considered by the British hop-grower.

Since the autumn of 1924 it has been reported also in Germany, France, Belgium and Russia.

After the first description of the disease in England(6), papers were published(7,8) which deal more fully with the history,

effect, and economic damage caused, and with the parasitism of the fungus so far as it had then been investigated. A widespread and somewhat alarming appearance of the disease took place in hop-gardens in Kent during 1925 and observation was made of the habits and progress of the fungus in relation to its host from the time of earliest growth until the hops were picked. In the course of this investigation, new facts in regard to the mode of parasitism of the fungus were discovered and short notes were published<sup>(9,10)</sup> concerning those details which were of practical importance, and a knowledge of which was likely to assist the grower.

One characteristic, though puzzling, feature of the disease is the production of more or less hypertrophied, "spike"-like shoots by the hop plant. These arise, commonly in company with other healthy normal bines, either from ground level (*i.e.* from the rootstock) or, later in the season, as stunted lateral branches at any height from the ground. They also occur at the tip of the main bine, which suddenly ceases growth and becomes "spike"-like, most commonly when it reaches a height of five to seven feet but sometimes at the top wire, twelve to fourteen feet from the ground. In the latter case and in that of the laterals, it is noteworthy that the remainder of the bine often appears perfectly healthy. On the leaves of such shoots, as well as on the surface of the stem, the mildew later produces its conidiophores in exceptionally dense masses often blackening the entire lower surface of the leaf.

The general appearance of the "spiked" growths suggested that they were due either to internal invasion by mycelium from the rootstock or to external infection by spores<sup>(8)</sup>.

The economic damage caused by the hypertrophy of the shoots, so far as it had then been observed, was described in a former article<sup>(8)</sup> as negligible. We now know, however, that a very large proportion of the bines trained up in a hop-garden may be affected and may cease growth at a height of about five feet. Further, as regards spread of the disease by nursery sets, the opinion was expressed that in addition to the ordinary means of dispersal of the fungus, mycelium might be transported within the stems of young plants. Not only has such been found to be the case, but "spike"-like shoots have also frequently been seen in nurseries attached to hop-gardens.

In the spring of 1925 the existence of hibernating mycelium in the rootstock was hypothetical and the investigations here described were undertaken with the object of obtaining information on this and on other points.

The earliest observed occurrence of "spiked" growths in 1925 was in the experimental garden at Wye on April 18th.

After that date they were frequently found both there and in commercial gardens in the county. In one instance a "spike" was found arising from the rootstock of a wild hop in a hedge and later in the season, both in Kent and in Devonshire, a wild hop was found with most of the laterals and main bines in the "spiked" condition. This indicated that processes of cutting adopted in cultivation, could not be involved in the question as to the causes of production of the "spiked" growths.

#### BASAL "SPIKES."

The first material collected on April 19th cast a doubt upon the hypothesis of external infection. Arising from the rootstock of one hop there was a short "spike" 4.7 cm. high which had three pairs of small curled leaves protruding, the fourth pair not yet free from the protection of the stipules and the fifth pair still enclosed, with the remaining leaves, in the terminal bud. Conidiophores and spores were present in great numbers on all the leaves and even on the stipules and could be seen also within the loosely folded terminal bud. Zoospores emerged from the conidia when these were placed in water. Close by, arising from the rootstock actually in contact with the "spike" described, there was a shoot, six cm. high, having two pairs of leaves expanded and a terminal bud. The whole of this was perfectly healthy and the internodes were longer.

Two other "spikes" on a different hop plant were next examined. In both cases they were removed, together with portions of the rootstock, and were found to be arising from points close to other healthy bines. In one, by means of hand sections, mycelium was found to be present in the cortex of the stem from the top to the bottom as low down as the bases of the small brown bud scales; in the other an attempt was made to trace it further and even into the older rootstock but it was found no lower than in the former case, though in this instance it occurred in the pith as well as in the cortex. These spikes were respectively ten and thirteen cm. high.

On April 26th a "spike" was cut, with a portion of the rootstock, from each of two different hop plants. In the first specimen a close examination with a lens and with a microscope of scrapings of the leaves (seven in number) showed that the fungus was present in the form of mycelium only, in five of the upper leaves (including those no more than projecting from the terminal bud) and that in two lower leaves there were hyphae and young conidiophores (sometimes unbranched) with immature conidia. In the stipules at the four nodes at which these leaves were inserted in pairs\*, only mycelium was found. Within the stem

\* One of the leaves of the lowest pair was missing.



Fig. 1.

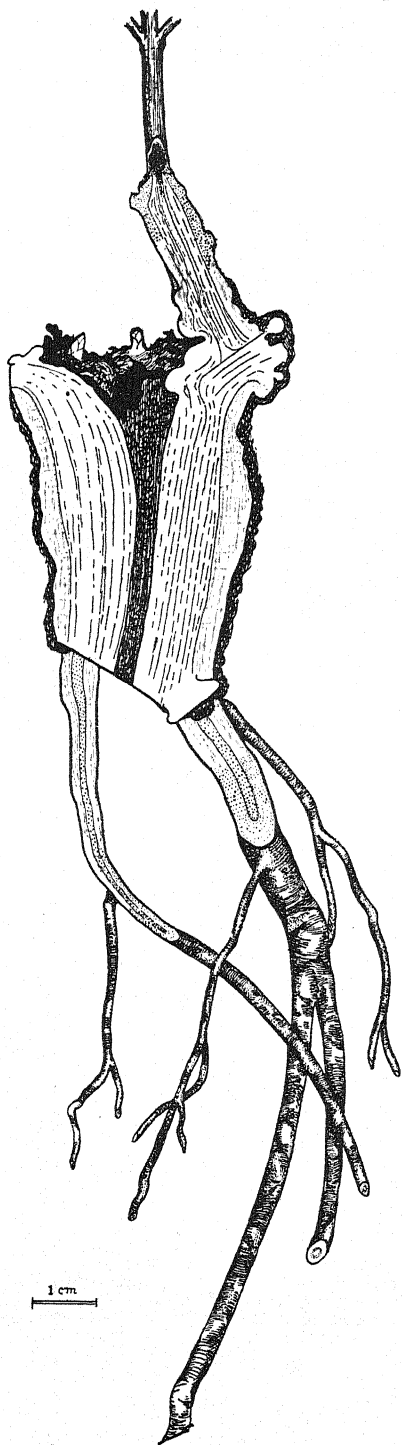


Fig. 2.

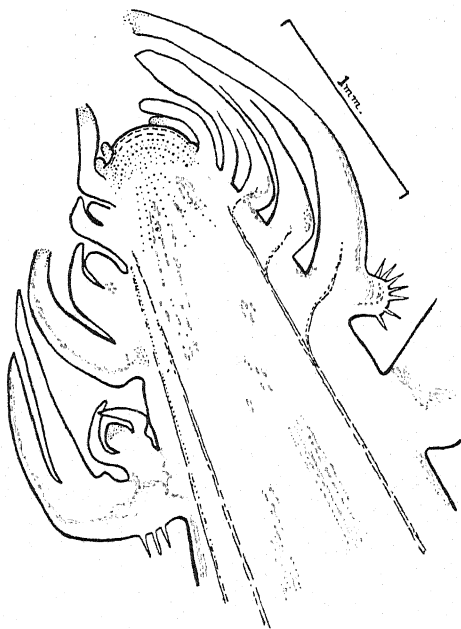


Fig. 3.

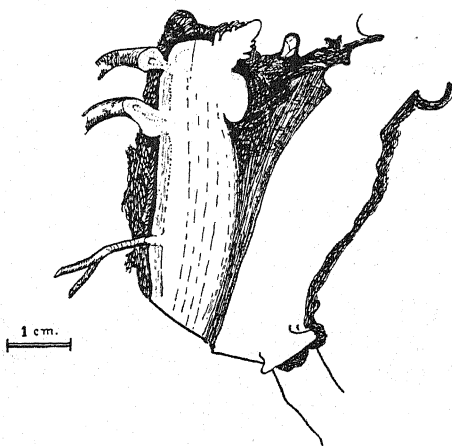


Fig. 4.



mycelium was present just above the junction with the older rootstock but could not be distinguished at any lower point. A longitudinal section cut half an inch below the node\* of the lowest pair of leaves revealed mycelium in the cortex running longitudinally and extending from close to the bast to within two cell layers of the epidermis, and in one case the hyphae were seen extending along the inner walls of epidermal cells. The whole width of the pith was also occupied by the hyphae.

A longitudinal section was cut in the petiole of one of the lowest leaves and part of the main stem above and below the junction. Hyphae were found in both cortex and pith of the stem, extending to the next internode above and thickly invading the petiole. A bud in the axil, cut longitudinally, showed heavy infection of all its scales to the very tip, though it was as yet not elongated.

Examination of the leaves and stipules of the second spike, by means of a lens, showed that although dense masses of conidiophores might be present on a pair of leaves at a node, they were not necessarily present also on the corresponding stipules. The reverse was also found to be the case. Stipules may be found brown and withered as though killed by the mycelium. Internally, hyphae were again found in pith and cortex and were present at the extreme base of the spike; in this and in one further case, mycelium was found at the junction with the rootstock.

The presence of mycelium in one-year-old portions of the crown or rootstock was not discovered until the middle of May when a rooted set bearing four short shoots, all of which were "spiked," was examined. One of these shoots was extremely short (one and three quarter inches long) having probably been eaten off by slugs†. Mycelium was present within all, and in the shortest, nor far from its base, nine oospores were found in the pith. It was observed in this and in subsequent cases, that where an accumulation of hyphae occurs, there is commonly a separation of the host cells and in some stems there are gaps of considerable size.

Hyphae having been found for the first time definitely below the actual stem of the shoot of that year, in the cushion of tissue from which the shoot and a number of dormant buds arise, the whole rooted set (of previous year's growth) was split with a knife and longitudinal sections were cut in several places throughout its length of three and a half inches. Mycelium was

\* This node was eight cm. above the junction with the rootstock and seven cm. from the tip of the terminal bud.

† The remaining three were five and a quarter, three and a half and three and a half inches long respectively and were not damaged except by the fungus.

found in abundance in pith, phloem and cortex and travelling radially in the medullary rays. The affected tissues were brown in numerous small areas (approximately three mm. diam.) and brown streaks were found in the wood. A break-down of the cortical cell walls was noticed.

One older set having portions representing growth of two previous years was examined and the mycelium was traced not only in the 1925 "spiked" growth, but also in the set of 1924 as far as its junction with the original 1923 set (*i.e.* one inch of woody 1924 growth).

These were the first two cases in which mycelium was observed in one-year-old tissue in addition to invading that of the current year's growth and they provided strong evidence that the mycelium was indeed perennial in the underground rootstock and had not merely invaded it in the course of the month or so during which active growth of the host plant had taken place above ground.

#### TERMINAL AND LATERAL "SPIKES" ON THE BINE.

The production of "spiked" growths at higher level, took place later in the growing season and seems to have occurred practically simultaneously in different parts of the county of Kent\*. During the second week of June, hops had reached a height of nine to twelve feet and plants attacked by the Downy Mildew were conspicuous by the fact that some, or all of the bines trained up had ceased growth after reaching a height of only five to seven feet. This phenomenon had been observed in previous years and has been described (7, 8), but an additional feature noted in 1925 was that bines stopped short in this manner often produce long and healthy laterals.

Bines were also observed which had apparently grown in a normal manner without any stunting at the tip, but which bore "spiked" laterals at one or more nodes.

For the purpose of investigating this stage of the disease whole bines were pulled from the hill and taken to the laboratory. Several were examined in this way and notes made as regards length, number of nodes, number and position of laterals, external signs of the fungus and its internal position. In some instances the terminal "spikes" themselves were cut off for examination with the microscope.

The first bine examined, which is typical of others, was that of a male hop, ten feet five inches long, and, at six feet two inches

\* In addition to their occurrence at Wye on June 10th, three enquiries on that day, concerning their sudden appearance, were received from growers in Kent who reported that only two or three days previously there had been no sign of arrested growth.

from the ground, there was one pair of "spiked" laterals (in this case male inflorescences) arising from the eleventh node. The rest of the plant, above and below this node appeared quite healthy, with the exception of a few angular spots on the leaves at the sixth node (one foot eight inches above ground).

The pair of laterals immediately below those noticed (at the tenth node) were nine inches long, whereas those diseased measured only two inches. At the next node above (twelfth) the healthy laterals were five and a half inches long. The internode above was eight inches and that below was ten and a quarter inches long. At the eleventh node itself, the petioles of the subtending leaves were thick and short, and there were yellowish patches on the upper surface of the laminae and corresponding areas black with masses of conidiophores, on the lower surfaces. These were the only two leaves on the whole bine to show such symptoms of disease. By means of sections cut longitudinally through the node mycelium was found in the pith, phloem and cortex of the main bine, in the petioles of the two leaves and in the lateral shoots in their axils. It was noticed that the extent of the mycelium was marked by a brown discoloration of the pith, which in healthy areas is pure white. By splitting the main bine, it was found that the brown discoloration extended 2.4 inches below the node examined and presence of mycelium to a distance of 2.0 inches, but no lower, was confirmed. The pith of the laterals and of the two leaf stalks was solid; that of the node nearly solid, *i.e.* with a very small passage at the centre, and that of the internodes hollow. Above the affected node the pith was brown and mycelium was traced to a distance of three and a half inches. Above this point the pith was white and no mycelium was found until the next node but one (thirteenth) was reached\*. Here again there was a brown discoloration, with mycelium, over a length of one inch below and one inch above the node. This node was at a height of seven feet six inches from the ground and thence upwards there was no mycelium though search was made through two feet ten inches of bine to the growing point at ten feet five inches from the ground. Sections cut longitudinally through the thirteenth node showed mycelium running in the pith and cortex of the main stem and into the pith of leaf petioles and of lateral shoots (male inflorescences), although neither of these as yet showed any outward sign of disease. In the lateral shoots mycelium was traced through four inches of their total length of five and a half inches but it apparently did not reach their growing points.

\* The internode between the twelfth and thirteenth nodes was the same length as the one below, *viz.* eight inches.

Several instances of a similar nature, in which isolated masses of mycelium are found in the neighbourhood of only one or a few nodes of a bine, have been met with.

As regards the terminal "spiked" growths, many of these have been examined internally and the case of one typical (female) bine may be quoted in detail. This was pulled from the hill on June 12th when it had become spiked and had ceased growth at a height of five feet eight inches. It bore sixteen nodes and a terminal bud. The lowest four nodes were without leaves or laterals, these probably having been stripped by the pullers, but at the fifth and up to the ninth both leaves and laterals were healthy. Above this there were no laterals. At the tenth node the leaves were healthy but at the eleventh, twelfth and thirteenth there was yellow mottling on the upper surface of the laminae; on the lower surface, near the junction of lamina and petiole, black masses of conidiophores were becoming apparent. At the fourteenth, fifteenth, and sixteenth nodes the leaves were smaller, mottled with yellow, but as yet no fungus was visible. All growth from the tenth node upwards constituted the "spike," the internodes of which were from a quarter to two and a quarter inches long, whereas those below that node were eight to ten inches long.

This bine was split from the base to the apical bud, and mycelium, accompanied by brown discoloration of the pith, was found continuous in nodes and internodes for a distance of fifteen inches, viz. from the apical bud to a point one and a half inches from the ninth node. At this node, and extending to the base, there was no mycelium, and the pith and cortex were white.

Sections cut in the infected part of the main bine (as well as in terminal "spikes" subsequently examined) showed hyphae particularly grouped in the region of the pith and where this was hollow masses of hyphae were found abutting on, or projecting into, the pith cavity. Such masses were approximately  $300\mu \times 300\mu$ .

In two cases papillate conidia and one or two conidiophores were seen amongst the wavy hyphae which were projecting into the central cavity of the pith. It was later discovered that the accumulations of hyphae were actually there in readiness for the production of oogonia, antheridia, and eventually of oospores, within the pith cavity. The first case in which mature oospores were found is described later. At certain places long hyphae, measuring up to  $900\mu$ , were observed growing into the cavity.

The above is an instance of what is of general occurrence, viz. the "spiked" bine is free from mycelium below the spike. On this account the advice was given(9) to growers to remove all

terminal "spikes" by cutting to a point at which the pith was white and to a node from which healthy laterals could be trained up in place of the main bine.

Variations from the typical example are sometimes found. Thus a bine with fourteen and a half inches of terminal "spike" obtained from a wild hop plant was four feet seven inches high, with twenty-two nodes; mycelium was found extending from the apical bud through sixteen internodes, to a point eleven inches from the junction with the rootstock, *i.e.* through a length of three feet eight inches. Occasionally it may be observed that a pair of leaves or laterals at a node which contains the mycelium may be free from attack; sections in such cases have shown that apparently the mycelium has failed to penetrate although it may be present in the stipules. On rare occasions one leaf at an infected node may be attacked by mycelium within petiole and lamina and bear black masses of conidiophores, whilst the other leaf at the same node may not be invaded and is healthy. In such cases, hyphae are plentiful in the pith of the node but are scarce or absent from the cortex on that side of the node at which the healthy petiole is inserted.

Infected stipules bearing conidiophores are of common occurrence wherever leaf infection is found. Where the leaves are still unfolded, as in the terminal bud, the stipules are sometimes covered with conidiophores before these appear on the leaves. In cases where lateral shoots are in the "spiked" condition, it may happen (as with a pair of leaves) that one of a pair at a node is quite healthy, having a thin stem and long internodes, whereas the other is invaded by hyphae and is stunted, having curled, infected leaves.

One notable example of a variation from the general appearance was in the case of a bine from a wild hop growing in the hedge and observed on June 14th. This bine terminated in a completely hypertrophied tip, nine inches long, with short rigid lateral branches of four nodes and having the leaves subtending the branches, suppressed or nearly so.

It sometimes happens that the tip of a bine may be in the "spiked" condition and yet may show no fungus fructifications on the exterior of stem or leaves. When such growths are split with a knife, brown discoloration of the pith or cortex is found and hyphae are present in these tissues. One such "spike," nineteen and three quarter inches (50 cm.) long, cut from the top of a bine, contained mycelium from just behind the growing point to a distance of fifteen and a quarter inches (39 cm.) down the bine, where, in the middle of an internode, the mycelium ceased and the pith was no longer discoloured. In the terminal bud itself, two millimetres long (fig. 3), hyphae were found  $250\mu$  from the

summit (apex) in the pith, and from the cortex they extended into both leaves and stipules.

Accumulations of hyphae, such as have already been described, were commonly met with in "spikes" the pith of which was hollow. They could even be distinguished with the naked eye when the stem of the "spike" had been split longitudinally. Although the pith is described as "hollow," several layers of pith cells are found within the vascular cylinder and these, being faintly coloured brown, tend to show up the mycelium which forms a white lining for the hollow pith. On June 14th, mature oospores were discovered. A terminal "spike" which had been kept in the laboratory for three days, when examined, was found to contain some hundreds of oospores in the pith—more especially in the pith cavity, where they appeared to have been produced on or by the accumulations of projecting hyphae. In some cases a clear oogonial wall was discernible and the diameter of this was  $40\mu$ – $50\mu$ . Mature oospores with thick colourless walls measured  $36\mu$ – $40\mu$ .

Many other cases of plentiful oospore production were found after this date in the pith of "spikes" freshly cut and not kept in the laboratory. Oospores have been found between seven and thirty inches from the tip of a "spike." In one instance they were found in a broken leaf petiole and in another at the base of a "spiked" lateral above its junction with the bine.

In no case could the nature of the antheridia be distinguished with certainty and attempts to induce germination of the oospores have not yet been successful.

#### THE HOP CONE.

As described in former articles<sup>(7,8)</sup>, damage of the greatest economic importance is caused by this fungus when it attacks the cones of the hop. During 1925 damage of this nature occurred not only in this country but also on the Continent. Cones which are in all stages of development, from four mm. ( $\frac{5}{32}$  in.) to five cm. (two inches) in length, and of a healthy green colour are liable suddenly to be turned brown in the course of a day or two.

During 1925 the first damaged cones were noticed on August 17th and, as on all subsequent occasions, angular spots caused by external infection were plentiful on the small cordate leaves which are borne on the branches of the female inflorescence close to the cones.

Conidiophores were abundant on the brown "petals" on both surfaces, though on the ventral they were more matted. By scraping or by examining the whole "petal" in a drop of water under the microscope, oospores were found (first on September

3rd) to be of common occurrence together with hyphae which ramified through the tissues. From this it is evident that the brown "petals" of affected cones can serve as a means not only of perpetuation but also of distribution of the disease. Cones discoloured through any cause are usually left unpicked in commercial gardens, with the result that "petals" (which are easily detached when ripe) may be carried considerable distances on the wind.

In view of the discovery of oospores in the cones, commerce in dried hops must not be lost sight of when considering means of dispersal of the disease. It is possible, however, that the process of drying may render any oospores incapable of germination\*.

#### HOP SETS.

The presence of mycelium within the basal "spikes" arising from the rootstock in spring having been determined, investigations were next made to ascertain whether the mycelium is capable of hibernating in the perennial underground portions of the hop plant.

The most convenient material for study was provided by young plants having "crowns" or rootstocks derived from cuttings ("cuts" or "sets") planted one or two years previously†. Cuts are planted in nursery beds during the winter or early spring. They are the swollen bases of bines of the previous summer's growth which have been cut from the parent rootstock. In the nursery bed, they form roots and above-ground stems during the following summer; in the succeeding winter they are known as "rooted sets" and may be used for planting out in the hop-garden. If, however, they are left undisturbed for a further season, the rootstock increases in size and the set is then known as a "stag."

During visits to gardens in 1925, nurseries had been observed in which the young plants were plentifully marked by the brown angular spots caused by external infection of the leaves. Nurseries were also known in which "spiked" growths had occurred amongst the tangled mass of normal thin bines, and from one such as this, sets were obtained on October 29th.

This is a usual time for the digging of "sets"; the leaves have fallen, and all above-ground stems have by then turned brown and dried up ("died back") and the "sets" may be regarded

\* In the process of drying in this country, the temperature does not exceed 140° F. when the hops are still moist and 160° F. when they are dry. The average time required is 9-10 hours. Sulphur dioxide is also used.

† The rootstocks of older plants in a hop-garden are very large and very tough and owing to the processes of "dressing" to which they are subjected, it is often impossible to distinguish the different years' growths.

as in the dormant winter condition. Several varieties were being grown in this nursery and five of these were especially chosen as having been heavily marked by the angular spot form of the disease on the leaves and as having shown a few "spikes" here and there in the rows.

The grower kindly supplied twelve sets of one variety and six of each of the others; choice of the sets, when dug, was quite at random, this being left to the hop-foreman.

The sets were washed, and it was observed that buds, in readiness for the spring of 1926, were numerous. These were in all stages of development up to one inch long and one-eighth of an inch thick\*. After drawings had been made in every case, the brown withered stems of the previous summer were split and examined but no mycelium or oospores recognisable as those of *Pseudoperonospora* were found. The sets were then split longitudinally with a sharp knife. In three of the varieties, none of the eighteen sets showed any internal discoloration of the pith, phloem or cortex of 1925 or 1924 growth and sections cut in each one indicated that no mycelium was present. Of another variety, only one of the six sets was discoloured internally, and by means of sections numerous hyphae were found in the cortex and phloem of that set but not in the other five. Heavy infection, accompanied by discoloration, was found in five of the twelve sets of the remaining variety. Thus, in six of thirty-six sets chosen at random, in early winter, the fungus was found and there is every reason to believe that such sets, infested with mycelium, may be the means of carrying the disease into any hop-gardens in which they are planted.

The first set examined had only a small fragment of the original 1924 "cut" attached and consisted therefore mainly of the swollen base of the 1925 growth. It was three and a half inches long, measuring from the dead base of the 1925 bine, and 0.6 inch in diameter. At the top were two buds—one inch, and three-quarters of an inch in length respectively—and others, somewhat smaller, were situated at various points. Mycelium was found thickly massed in the cortex, phloem and medullary rays of the main stem. It was present at the bases of the buds and reaching as far as the brown bud-scales but in no case were hyphae found penetrating a bud.

There were eight roots, some of which were over eight inches long and 0.3 inch in diameter and it was noticed that some were tinged with brown for a short distance from their junction with the set. A median longitudinal section of the set showed that

\* Early production of buds underground is found in the case of normal healthy hops. In 1925, buds up to half an inch long were observed on October 19th.



the cortex and phloem on both sides were discoloured throughout. The presence of mycelium in all the pale brown areas was confirmed and hyphae were also found (wherever there was brown coloration) permeating the cortex of the roots over an extent of one inch to one and a half inches from the set.

The other five sets had each a considerable length of older tissue. Mycelium was found in one of these extending throughout three and a half inches of the 1924 growth but the base of the 1925 bine had died back instead of thickening and it was therefore impossible to trace the mycelium into this. In the second, mycelium was found in the base of one of the 1925 bines (where it was massed in proximity to several buds) but it was not found in the 1924 tissue. In the third, it was present not only in the swollen base of the 1925 bine, but also in the cortex and phloem of the original 1924 set and was traced over a distance of three inches. The fourth and fifth sets were not split with a knife but small wedges were removed by cutting through the outer corky layer. Samples of the tissue were thus secured from the entire length of the sets and mycelium was found infesting the older (1924) parts throughout a length of four inches and one and a half inches respectively. In only one branch of one of these sets were hyphae found in the base of the 1925 growth also.

By not causing any serious damage to these two sets in the course of their examination they were kept in a state fit for further growth and after having been sketched and the position of buds and of mycelium noted, they were planted for further observation at a time when the buds are rapidly elongating.

Five further cases of invasion of the roots by mycelium were met with; hyphae were traced as far as two inches into the roots, of which some were nineteen inches long and otherwise healthy. A dark, almost black, coloration was noticed on the outside of roots which proved to be infected. This marking covered only the extent of the worst infestation and was not more than one and a half inches long.

Some alteration in the consistency of the phloem takes place in the presence of the mycelium. When the set is cut, even with a sharp knife or razor, an infected phloem is found to be more spongy and tears instead of being readily cut. It would appear that the fibres are no longer held in place when the medullary rays are infested. Hyphae were observed only once in the cushion of tissue on which buds are formed and never at that time of year were they seen to have entered a bud.

## METHODS.

In following the course of the mycelium in the living plant, specially at a time of the year when it was unaccompanied by any form of fructification, reliance was placed upon its general characteristics and on certain circumstantial evidence.

The great size of the hyphae—both as regards breadth and length, their generally unseptate condition, their apparently vigorous growth with occasional formation of a definite group or flat plate, their intercellular habit and, lastly, their characteristic haustoria were the principal features relied upon.

Within the pith or cortex of the "spiked" growths and of the bines, the hyphae are readily distinguishable in hand sections without the use of stains, but in the dense young tissue of a terminal bud and especially in the tough fibrous tissue of one- or two-year-old "sets" they were found extremely difficult to differentiate.

By the use of Azo Blue stain for all sections, the mycelium and haustoria were made readily visible. A one per cent. solution was made slightly alkaline by the addition of caustic soda until the colour was decidedly red. Sections of fresh material were cut and transferred directly to a drop of the stain on a slide. Parts of the hop plant being presumably acid, some sections tended to restore the blue colour of the stain; to prevent this, a small glass rod, previously dipped into a weak solution of caustic soda, was, when necessary, brought into contact with the drop of stain and the red colour, which gave the best results, was again obtained.

Both the host tissues and the hyphae became stained, but the latter more deeply than the former. Haustoria were specially well defined. Where the hyphae were few or did not readily stain, the sections were left for one or two hours in the stain on the slide; as a rule, however, staining was immediate.

## CONCLUSIONS.

The principal fact brought to light by the investigations of 1925 is that mycelium of the fungus may persist during the winter in underground portions of the hop plant. Similar behaviour by species in four genera of the Peronosporaceae has been recorded between 1861 and 1915 by various authorities, and their observations have been summarised in a paper by Melhus<sup>(4)</sup>, who has himself contributed five such records.

Since that date, further examples or confirmation of the existence of a perennial mycelium in the Peronosporaceae have been provided; in 1920 by Gardner<sup>(2)</sup> in the case of *Peronospora parasitica* in turnips; in 1921 by Murphy<sup>(5)</sup> in *Peronospora*

*Schleideni* in onions and shallots; and in 1925 by Klebahn(3) in the case of *Peronospora pulveracea* in *Helleborus* sp.

In the present fungus, it has not yet been possible to connect the mycelium found in the underground parts, with the fruiting stage either by pure culture or by exposing the cut surface of "sets" to a damp atmosphere. There can be little doubt, however, that the Downy Mildew does indeed over-winter in the rootstock and the inference is that in early spring it probably invades one or more of the developing buds, apparently either overwhelming the young shoot—in which case a basal "spike" would result—or is carried by the rapidly elongating bine close behind the growing point. In the latter case it would appear from the sections made that the mycelium may either be carried up to a considerable height in its entirety or small quantities may be deposited at certain nodes in the course of the rapid upward growth of the bine. This procedure, if it takes place, would account for the formation of diseased laterals at isolated nodes and of terminal spikes at any height from the ground.

It must be pointed out, however, that up to the present the actual passage of the mycelium from the rootstock into an elongating bud has not been observed.

Whether "spiked" growths may be caused by external infection, an alternative suggestion put forward in an earlier paper(8), still remains to be proved. Professor Ducomet(1), in an account of his observation of the disease in France, is of the opinion that early external infection alone takes place and that this is sufficient to account for all the forms of "spiked" growths.

The above work has been carried out in co-operation with Professor E. S. Salmon, with whom visits were paid to numerous hop-gardens where the necessary material was obtained. The author desires to express his thanks for these and other facilities.

#### SUMMARY.

An account is given of the discovery and investigation of an internal invasion of the Hop (*Humulus lupulus*) by the Downy Mildew *Pseudoperonospora Humuli* (Miyabe et Takahashi) Wils.

Hypphae are present within the cortex and pith of the stem of "spiked" growths from the rootstock and are also present in one-year-old parts of the rootstock. In the case of longer bines with terminal or lateral spikes, the mycelium is found within the bine but is not necessarily continuous and healthy intermediate nodes and internodes may occur. Mycelium has not been observed at the base of such longer bines.

The fungus is present in the winter in the pith, phloem and cortex of nursery "sets" and in several cases has been found in the cortex of the roots.

Oospores occur plentifully in the pith of terminal and lateral "spikes" and within the bracts and bracteoles of the "cone."

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# EXPLANATION OF FIGURES 1-4.

- Fig. 1. Downy Mildew of the Hop: Portion of a rootstock showing one healthy and two diseased shoots in the form of basal "spikes," with small, curled, grey-green leaves. In the "spikes," mycelium was present, and in this case was traced only as far as the junction with the rootstock. The healthy shoot (left) is distinguished by its longer internodes, sinuous growth, and normally expanding dark green leaves. April 27th, 1925. Natural size.
- Fig. 2. A nursery set (in section) derived from a cutting planted in 1923, and examined November 11th, 1925. Mycelium of *Pseudoperonospora Humuli*, the position of which is diagrammatically represented in red, was found in the bast and cortex of the older parts and also in the swollen base of the 1925 growth. In addition it was present in the roots. 0.8 Natural size.
- Fig. 3. Diagrammatic outline drawing of a freehand section cut longitudinally through the apical bud of a bine with terminal "spike" at about seven feet from the ground. The position of mycelium is represented in red. From here, hyphae were traced downwards to a distance of 39 cm. in the bine. x 28.
- Fig. 4. Section passing through a bud on the older portion of the set shown in fig. 2. Examination of sets in winter (November) indicated that buds were not invaded by the fungus. Position of mycelium shown in red. 0.8 Natural size.

## HETEROTHALLISM IN THE GENUS PENICILLIUM.

### A PRELIMINARY NOTE.

By Dr H. G. Derx.

THE occurrence of ascocarps in the genus *Penicillium* is rather uncommon, reproduction taking place as a rule by means of abundant conidia. In some cases, however, ascocarps have been found and described more or less adequately, but it is usually impossible to corroborate investigations of older authors, either because their strains are no longer obtainable, or because it would seem that some species of *Penicillium* lose the faculty of producing asci after a certain number of transplantings on artificial media.

A study of the different perithecia described in this genus leads to the conclusion that they differ to such an extent that it would seem impossible to unite all species in one genus. Some species form perithecia resembling those of the genus *Gymnoascus*; others should be placed in the genus *Eurotium*; and yet others seem to form asci in the interior of sclerotia after a resting period which may be considerable.

So far I have never seen perithecia formed in the last two ways; sclerotia sometimes appeared but asci never developed in them. I have, however, in pure culture four species of *Penicillium* the perithecia of which closely resemble those of *Gymnoascus*, with the only difference that the enveloping hyphae of the ascocarp are more closely interwoven than is usual in that genus.

These ascocarps are formed with great regularity and, in certain circumstances, so abundantly as to crowd out the conidial form almost completely.

At present I have the following species in culture:

1. *Penicillium Wortmanni* Klöcker (1903), kindly sent to me by Professor Ph. Biourge, who obtained it from Klöcker.
2. *Penicillium vermiculatum* Dangeard (1907). This curious species was described in detail by Dangeard, but has escaped the attention of specialists. Besides a strain isolated by myself I possess that described by Thom as *Penicillium luteum* (Washington, No. 11), which is identical with this species.
3. *Penicillium luteum* A. Richards (1924). The name of this must be changed. It is quite different from *Penicillium luteum* Zukal (1893) and from *P. luteum* Thom mentioned above. I obtained it from the Centraalbureau voor Schimmelcultures at Baarn (Holland).

4. *Penicillium luteum* (Zukal?) Wehmer *certissime*. This is the species in which I discovered heterothallism. It is the only *Penicillium* in my collection which agrees in all points with C. Wehmer's description of *Penicillium luteum* Zukal.

I am very much obliged to Dr Ch. Thom for sending me five other strains of ascogenous *Penicillia*, which with those mentioned above will form the subject of a further communication in which their characters and especially the development of perithecia, etc., will be described in detail. I hope that other workers will kindly send me any ascogenous *Penicillia* they might possess or isolate as it is only by such aid that a more or less complete knowledge of the ascocarps in the genus *Penicillium* can be reached.

Numerous different species have been named *Penicillium luteum*. Almost every mycologist has a *Penicillium* of this name in his collection, but few investigators have ever seen the typically formed ascospores as figured by Zukal. Yet many authors, when describing their *Penicillium luteum*, content themselves with referring to Zukal for a description of ascocarps.

According to Zukal the asci are formed in perithecia consisting of closely interwoven hyphae, which are at first yellow, then orange, and which at last turn blood-red.

The asci are stalked; the ascospores are oval,  $4.8\mu-3.3\mu$  with four verruculose, tricostate ridges. The conidiophores typically produce a verticil of metulae, each of which is crowned by a verticil of conidiiferous cells. The conidia are blueish-grey.

The only author who described ascospores which could be mistaken for those of Zukal's *Penicillium luteum* is Wehmer, who in 1893 described a mould which he stated was extremely common, and which he considered identical with *Penicillium luteum* Zukal. The ascospores of Wehmer's mould certainly have three or four cross-ridges, which are however perfectly smooth, instead of being verruculose. One might possibly attribute Zukal's account of wart-like prominences on the extremely fine ridges to optical illusion due to imperfection of the microscopes in his day, but this suggestion remains to be proved.

I therefore believe that the mould which I found to be heterothallous, and which presents all particularities described by Wehmer, should be named provisionally: *Penicillium luteum* (Zukal?) Wehm.

The orange-red perithecia of this mould were found growing on the sand of a dried-up aquarium and contained ascospores closely resembling those drawn by Wehmer. The perithecia were crushed and planted on cherry-juice agar. A *Penicillium* developed, which produced, besides abundant perithecia, the biverticillate conidiophores as described by Wehmer. These

characters repeated themselves in subsequent transfers on the same medium, but the quantity of perithecia produced diminished constantly, and with fear I foresaw the moment, that—in close accordance with the experiences of Wehmer—they would no longer be produced at all.

I tried to purify the original culture by the dilution method in Petri dishes. Several strains were isolated, which, though morphologically identical, presented some differences in physiological characters. Some strains, on Raulin-Dierckx gelatin at 20° C., produced dark olive-green conidia; the reverse of their colonies was of a brilliant orange colour, which did not diffuse into the gelatin. The medium was liquefied after some days, and large quantities of minute crystals (calcium oxalate) were formed, which clouded the liquefied gelatin. Other strains, on the same medium, produced blueish grey-green conidia, the reverse, instead of being dark orange, only showing patches of a currant-red, diffusible colour. Gelatin was liquefied slowly, without clouding, but finally becoming deep red in colour.

None of the strains thus isolated ever produced perithecia, whereas, on the other hand, ascocarps were formed after some weeks in the original Petri dishes.

Thus there remained only one way to obtain the ascogenous *Penicillium* with certainty, *i.e.* to make single ascospore cultures.

With the aid of the apparatus of Janse and Péterfi I succeeded in obtaining twelve such strains, each grown from a single ascospore. They were identical with the strains isolated by the dilution method, and showed the same physiological differences. But not one perithecium was formed. At the most yellow mycelial masses, closely resembling perithecia, were formed in some strains, but these masses were quite devoid of asci. It is now clear to me that these empty masses are "haploid ascocarps" comparable to the haploid fructifications of *Coprinus fimetarius* described by Brunswik.

These mono-ascospore cultures, however, readily and abundantly produce well-developed ascocarps when they are grown two by two in certain combinations. When this point had been fixed, the different strains isolated from conidia behaved in the same way.

Thus, the heterothallism of *Penicillium luteum* (Zukal?) Wehm. is beyond doubt.

Table I shows the result of the crossing experiments of the twelve mono-ascospore cultures in all possible combinations. It will be clear that the strains may be divided in two groups. There are (+) strains: *B*, *C*, *H*, *I*, *L*, *M*; and (−) strains: *A*, *D*, *E*, *F*, *G*, *K*. It goes without saying, that the choice of the signs (+) and (−) for either group is at present absolutely

arbitrary; only a cytological examination would enable one to decide whether a certain sign is proper to one group rather than to the other.

Table I.

Mono-ascospore strain	A	B	C	D	E	F	G	H	I	K	L	M
A	—	1	1	—	—	—	—	2	1	—	2	1
B	1	—	—	2	3	3	1	—	—	1	—	—
C	1	—	—	2	3	3	1	—	—	1	—	—
D	—	2	2	—	—	—	—	3	2	—	3	2
E	—	3	3	—	—	—	—	4	3	—	4	3
F	—	3	3	—	—	—	—	4	3	—	4	3
G	—	1	1	—	—	—	—	2	1	—	2	1
H	2	—	—	3	4	4	2	—	—	2	—	—
I	1	—	—	2	3	3	1	—	—	1	—	—
K	—	1	1	—	—	—	—	2	1	—	2	1
L	2	—	—	3	4	4	2	—	—	2	—	—
M	1	—	—	2	3	3	1	—	—	1	—	—

The numbers indicate the relative quantity of perithecia developed:

—=none. 1=very feeble. 2=feeble. 3=good. 4=abundant.

The strains *E*, *F*, *H* and *L* show sterile, haploid ascocarps.

Table I also shows that in both groups one may remark distinct differences in sexual activity. For instance, the strains *A* and *G* only form perithecia abundantly with the strains *H* and *L*. Thus the strains *H* and *L* show the greatest sexual polarity of the (+) sign. They are more active than the strains *B*, *C*, *I*, and *M*. Among the (−) strains, the cultures *E* and *F* possess the largest degree of sexual polarity. The strains *A*, *G* and *K* are only feebly "polarized." Further, the strains of great sexual polarity in *both sexes* are capable of forming *sterile* haploid ascocarps without being crossed.

In this connection it is very interesting to remark that there would seem to exist some parallelism between the degree of sexual polarity and some purely physiological characters. The strains quickly liquefying gelatin and of which the reverse on Raulin-Dierckx gelatin is deep orange are among the most active in their sexual reaction (*B*, *C*, *E*, *F*, *H*, *L*). The strains *A*, *D*, *G*, *I* and *K* liquefy gelatin only slowly, with production of the red diffusible colour; their sexual activity is at the same time rather feeble.

The question arises whether the sexual activity remains constant after several transplantings. It will be equally interesting to establish whether it appears unchanged in the  $F_1$  generation. Also the problem must be solved, whether the physiological differences of the strains are due to true mutations or whether they are merely degenerative modifications (mutations!).



Cytological researches must first be made to elucidate the manner in which the sexually different thalli fuse, the way in which gametophores and ascogonia are formed, and the cytological phenomena of fusion and ascus formation.

*Penicillium luteum* (Zukal?) Wehm. furnishes the first example of heterothallism in this common genus.

Certainly it will not remain unique for long. In fact most of the strains available in collections are the result of repeated purification by the dilution method, so that there is some chance that they are derived from single conidia. Others have been found more or less accidentally in air-dust. Modern methods of purification have certainly contributed to the relative rarity of ascocarps observed recently, so that it would be of some interest to cross strains of the same, or of morphologically similar moulds occurring in different collections.

I wish to express my thanks to Professor Dr A. J. Kluyver for the hospitality I have enjoyed in the Laboratory of Microbiology of the Technical University at Delft and to Mr C. B. van Niel, curator of this laboratory, for his kind and indispensable aid in the isolation of single ascospores with the apparatus of Janse and Péterfi.

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### ON THE OCCURRENCES OF A SPECIES OF COLLETOTRICHUM.

By W. Small, M.B.E., M.A., B.Sc., Ph.D., F.L.S.

#### INTRODUCTION.

It has been the experience of the writer to find in the course of his general mycological work in Uganda that forms of the genus *Colletotrichum* obtrude themselves upon his notice more frequently, perhaps, than forms of other equally widely distributed tropical plant-inhabiting fungi, and, in any case, so frequently that they compel a certain amount of attention. It is the purpose of this paper to relate some of the occurrences of a species of the genus and the experimental work conducted with the various isolations of the species in question. The study was

undertaken in order to establish or, at least, throw light upon the local status of the various forms encountered and the nature of the species which can be found on so much diverse material. In passing, the writer wishes to make a plea for similar studies of similar fungi occurring in other parts of the tropics. By "similar fungi" is meant such forms as occur on many different substrata, have among themselves a strong morphological likeness but a less well-marked habit of parasitism or saprophytism, and occur in a definite restricted area. The accumulated data from such work would be of great and increasing importance as their significance was gradually extended beyond their original local limits by the linking up and correlation of results and conclusions.

The writer has been accustomed to refer to the species of *Colletotrichum* which forms the subject of these notes as *C. coffeanum* Noack. Since other specific names of *Colletotrichum* are as applicable as *coffeanum* on morphological grounds, and one of them at least, *incarnatum*, was described by Zimmermann from cacao pods in the same year (1901) as *coffeanum*, and another, *Camelliae*, was instituted by Massee as early as 1899, *coffeanum* was adopted not for reasons of priority. It was, however, the name given to the first *Colletotrichum* encountered by the writer in Uganda, and, as it then occurred on *Coffea arabica*, it was natural to refer to it as *C. coffeanum* and to apply the same name to forms which, although they occurred on host plants other than coffee, were morphologically indistinguishable from the original *C. coffeanum*. The name *coffeanum* has thus been retained primarily for reasons of convenience, and, since it has been shown that many of the forms labelled *coffeanum* in these notes are characterised by the perfect stage *Glomerella cingulata* (Stonem.) Spauld. and v. Schr., and that that name should therefore be used in reference to them, the actual designation of the conidial stage is a matter of minor importance. In describing the species from leaves and twigs of coffee in Brazil, Noack gave the conidial measurements as  $12-18 \times 4-5\mu$  and the diameter of the acervuli as varying from  $\cdot 15$  to  $\cdot 18$  mm. The pink or flesh-coloured masses of conidia are distinctive, and *coffeanum*, as it is found on coffee in Uganda, either causing what is known as brown blight of leaves and berries or living saprophytically on dead or dying branches, corresponds in the main to these measurements. Noack gave no measurements of the setae often present on the acervuli of *C. coffeanum*, but Butler's "four or five times as long as the spores" is sufficiently accurate for practical purposes\*, although setae up to  $180\mu$  in length have been found on material kept in the laboratory. The

\* Fungi and disease in plants, 1918, p. 482.

presence or absence of setae is well known to be a variable character in *Colletotrichum*, and it is now generally recognised that the genus *Gloeosporium* is, at least in part, only a *Colletotrichum* without setae. No distinction is made here between the two genera, and the generic name *Gloeosporium* is not used, although it is in many cases as applicable as *Colletotrichum*. In the various occurrences of *C. coffeanum* listed below, the most constant morphological features of the species are the conidial sizes, the pink coloration of the conidial masses and the dimensions of the acervuli, and, on these features alone, the placing of the different forms in *C. coffeanum* is justified. Less rigid morphological characters are the presence or absence of setae and the presence or absence of the ascomycetous or *Glomerella* stage of the fungus. Setae are most commonly present on a dry or drying substratum both in nature and in artificial culture, and, while their presence or absence has thus been found to be in part a response to environmental conditions, it cannot be said that such conditions are entirely responsible for their development or non-development or that they can be produced or suppressed at will. At the same time, observations on several generations of different isolations of the fungus grown on half-a-dozen different media have not shown that any of the strains possesses an innate or heritable tendency towards setal production, and the development of setae would therefore seem to be controlled by arbitrary or even accidental factors. In the case of the perfect stage of the *Colletotrichum*, the production of perithecia under laboratory conditions has not been proved to depend on the substratum, whether natural or artificial, and it seems as if the opinion expressed by Dastur\*, that the perithecial-forming faculty is the outcome of some quality inherent in the strain of the fungus, holds in Uganda.

#### THE HOSTS OF *Colletotrichum coffeanum*.

The following list of occurrences of *C. coffeanum* in Uganda is representative but by no means exhaustive. Notes on each case are appended, and it will be observed that the fungus is at times apparently parasitic and at others palpably saprophytic. On the whole, my impression is that the tendency of the *Colletotrichum* under discussion towards saprophytism or weak parasitism is stronger than its leaning towards obligate parasitism. *Colletotrichum coffeanum* has been under observation in the field for more than ten years and there is no evidence of an increasing amount of growth on the part of the fungus on, for example, coffee, a plant which it has had unlimited opportuni-

\* Ann. Appd. Biol. vi (1920), 245.

ties of attacking. Among the plant diseases caused by *C. coffeanum* in Uganda, none approaches in virulence the diseases of citrus caused in Florida by *C. gloeosporioides* (Penz.) Sacc., the anthracnose of cotton caused by *C. Gossypii* Southw. in the United States of America, or the anthracnose of beans due to *C. Lindemuthianum* Br. and Cav., despite the fact that these species of *Colletotrichum* are of the same morphological type as *C. coffeanum*. It was stated by Noack himself with regard to *C. coffeanum* in Brazil that the fungus did harm only to weakly trees, and there is much field evidence in Uganda in favour of the view that, other things being equal, the healthy plant has less chance of being attacked by *Colletotrichum* than its sickly or overburdened neighbour.

Identification of the fungus is based upon the more constant morphological details mentioned above, for they, as criteria, must assume a greater importance than usual in the case of a species which not only occurs on many different substrata but also has no definite and constant habit of growth. Also, the identification of the *Colletotrichum* on the various natural substrata has been supported in every case by cultural comparisons so that there might be no doubt of the accuracy of the naming. It has been shown by various writers, and more recently by Dastur\* and Burger†, that variations within species of the genus *Colletotrichum* take place in culture, and it has been found that, apart from the question of the identity of the species studied by these authors with the species *C. coffeanum*, similar variations occur in Uganda. Purely morphological studies, however, have been subordinated to the physiological side of the work on *C. coffeanum*, and, while they are reported in these notes, they have not been pursued as fully as their importance warrants. More time and attention have been given to the inoculation and cross-inoculation studies which are discussed later.

1. *Coffee*. Brown blight of coffee (*C. arabica*) leaves and berries has been known in Uganda since the beginning of *arabica* cultivation on a large scale about fifteen years ago, and *C. coffeanum* has been proved to be the cause of it. The same fungus occurs on the branches of trees in the desiccated condition known locally as "dieback," but it has been shown to have little or nothing to do with bringing about this which is really due to mal-nutrition caused by a complex of physical and physiological factors‡. It follows also in the wake of certain sucking

\* Loc. cit.

† Jour. Agric. Res. xx (1921), 723.

‡ Circs. Nos. 4 and 9, Dept. of Agric. Uganda, 1920 and 1923; Kew Bulletin, 1921, p. 57.

insect pests that kill back young shoots, for example, the capsid bug *Lycidocorus mimeticus* R. and P. In fact, the conidia of *C. coffeanum* are so commonly present on coffee in the field that a large proportion of healthy green twigs and leaves plucked at random and placed in a damp chamber for a few days without surface-sterilisation will yield acervuli of *C. coffeanum* on both wood and leaves, and it follows that, if *C. coffeanum* were a consistently aggressive parasite of coffee, the results of its presence would be disastrous. On the contrary, brown blight is not a very common trouble, and it has never assumed the proportions of an epidemic. The same *Colletotrichum* occurs on *robusta* leaves, berries and twigs in circumstances similar to those under which it is found on *arabica*, and it has been noted in association with *Cephaleuros Mycoidea* Karst. on twigs, leaves and berries of *liberica* coffee. The fungus may therefore be found on coffee as a parasite or as a saprophyte. The perfect stage has been obtained on *arabica* twigs and in culture from several strains and has been identified as *Glomerella cingulata*.

2. *Cacao*. A *Colletotrichum* indistinguishable morphologically from the form on coffee has been obtained on several occasions from rotted and hardened cacao pods and from apical twigs which had died back. Certain strains of the cacao fungus were tested in 1919-20 and found to be only wound parasites of cacao. The same strains, however, infected coffee leaves. They developed the same *Glomerella* stage as the coffee strains, and could therefore be safely named *C. coffeanum*\*.

3. *Cotton*. *C. coffeanum* is frequently found on bolls in association with a discoloration which resembles the anthracnose caused in other regions by *C. Gossypii* Southw. No doubt true anthracnose is present in Uganda, but a boll discoloration which is not true anthracnose is quite common. It is probably caused by the sucking of insects, and the fungus associated with it may therefore be a saprophytic follower of injury brought about by another agent. Attempts to induce the fungus, recently isolated from a discoloured boll, to penetrate the unbroken skin of a healthy boll have failed, but when the fungus is provided with a passage through the skin of the boll in the form of needle-prick wounds it is able to live on the boll tissue and contents and cause or initiate a kind of internal rot.

An anthracnose of cotton seedlings which agreed in its symptoms with a similar trouble in the United States of America† was found for the first time in Uganda in 1924. Many of the affected plants recovered from the disease, but they did not make full normal growth and they bore a reduced number

\* Kew Bulletin, loc. cit.

† Farmers' Bull. 1187 (March, 1921), 15, fig. 10.

of bolls at maturity. The only fungus isolated from the diseased tissue was *C. coffeanum*. Inoculation experiments were attempted by three methods. In the first, cotton seedlings and the *Colletotrichum* were grown together in large tubes in dilute Richards' solution, but, as the medium could not be kept free of contaminations and the control plants did not thrive, the results were invalidated. In the second, cotton seedlings were grown in tins of sterilised soil into which, among the roots of the plants, large numbers of viable conidia in sterile water and in sterile weak cane-sugar solution were injected by means of a small syringe. Both control and inoculated plants grew normally, and results were therefore entirely negative. It should be added that the seeds used in the foregoing experiments were delinted with sulphuric acid and surface-sterilised before germination. In the third method, both delinted and sterilised and untreated fuzzy seeds were used. Inoculation was attempted by dipping the seeds in water containing conidia and immediately planting them, by a similar dipping followed by an incubation period of forty-eight hours in a damp chamber before planting, and by placing drops of water containing conidia on needle-prick wounds, a process which was followed by incubation for forty-eight hours before planting. The first two sets of these latter experiments gave negative results with both delinted and fuzzy seed, and the third lot gave positive results only in the case of the delinted seed. The resulting anthracnose, however, was more severe than the field disease, for it attacked eighty per cent. of the plants and proved fatal to more than half of them. The results point to the necessity for the presence of the fungus (at least of the strain employed) within the seed as a preliminary to the causation of seedling anthracnose, and they also disclose the possibility of the passing of the *Colletotrichum* from an apparently saprophytic condition on a boll to a parasitic state on young seedlings via the seed, a point of practical (and theoretical) importance which the writer hopes to investigate more fully during the next cotton season.

4. *Beans.* An anthracnose of the pods of French or kidney beans (*Phaseolus vulgaris* L.), the symptoms of which are identical with those described from other areas, occurs at times, and the *Colletotrichum* associated with the disease is indistinguishable from *coffeanum*. It has been proved to be capable of causing the anthracnose of pods. The *Glomerella* stage of the fungus has not been obtained, nor has the affection caused by the *Colletotrichum* been found on any part of the plant but the pods. In 1924, *C. coffeanum* occurred on the stems of a small proportion of a number of native beans (*Phaseolus* sp.) which had been wilted by an attack of *Sclerotium bataticola* Taub. during

a spell of hot dry weather, but it was shown to be an inactive agent in the production of the wilt through the soil\*. The *Glomerella cingulata* stage of this strain was found in old dried cultures.

5. *Tea*. *C. coffeanum* has been found upon the leaves, twigs and seed-vessels of tea plants grown for seed purposes. The leaf blotch which it causes resembles the brown blight of coffee, and it is often caused by the *Colletotrichum* alone; that is to say, unaccompanied by other fungi like *Pestalozzia*. The extent to which the fungus is responsible for injury to branches or seed-vessels has not been determined, but a recent isolation from leaf-blotch has been proved to be able to blight a small proportion of the leaves of young plants when brought into contact with their upper surfaces. The relation of the fungus to the tea plant is probably of the same nature as it is to coffee, and its perithecial stage is *Glomerella cingulata*.

6. *Citrus*. *C. coffeanum* has been encountered on orange leaves, where it has an effect similar to that on coffee and tea leaves. A few upper-surface inoculations on the leaves of young orange plants have been successful in producing a browning resembling that which occurs in nature, but infection is slow. The period required to produce the leaf blight is three or more weeks.

7. *Chillies*. A ripe rot of the fruit of a local *Capsicum* (probably *frutescens* L.) is caused by *C. coffeanum*. The fungus has been recorded only on the ripest fruits, and it has not been noted to cause an anthracnose as distinct from a ripe rot. The trouble, however, is not common.

8. *Hevea brasiliensis* Müll.-Arg. The fungus has been found on leaves and small dieback twigs of the Pará rubber tree. It has not been seen on the fruits. While it may cause defoliation on a small scale, as on young plants in laboratory experiments, there is no evidence that it does so on a large scale in the field. Its presence on twigs is probably a danger inasmuch as it may lead to attacks of the dieback fungus *Botryodiplodia Theobromae* Pat.

9. *Mango*. *C. coffeanum* causes an anthracnose or spotting and a ripe rot of mango fruits. Both affections have been reproduced under laboratory conditions, but, in recent work, a ripe-rot strain of the fungus could not be induced to penetrate the unbroken skin of green unripe fruit. It is not known with certainty whether the fungus also causes an anthracnose of floral branches and whether it infects the fruits in their younger stages. *C. coffeanum* was obtained several years ago from the floral branches of a tree which failed to set its fruit, but its con-

\* Kew Bulletin, 1925, p. 118.

nection with the trouble was not proved. *Glomerella cingulata* has been developed on pieces of skin removed from successfully inoculated fruits and kept in a dry place under cover.

10. *Avocado pear* (*Persea gratissima* Gaertn. f.). Black spots are common on the fruits of avocado, and *C. coffeanum* can be isolated from the diseased tissue. The fungus is also associated with a ripe rot of the fruit. Black spot has been reproduced only through needle-prick wounds, a result that agrees with the finding of Stevens in Florida\*. *C. coffeanum* has been found at times also on small branches which have dried up, but it was not shown to cause a dieback in a series of tests in 1922. *Glomerella cingulata* has also been obtained on or near the sites of old *C. coffeanum* acervuli on the branches.

11. *Soursop* (*Anona muricata* L.). *C. coffeanum* has been isolated from dieback twigs and spotted fruits of the soursop, but no experimental work has been carried out with this host.

12. *Papaw* (*Carica Papaya* L.). An anthracnose of flowers and young fruits such as is described by Dastur† has not been recorded in Uganda, but *C. coffeanum* has been found several times in association with darkened depressed areas of fruits which are about to change colour and ripen. The affection of such fruits spreads until it becomes a general rot. A recent strain of the fungus isolated from a depressed area of a green fruit was found to be capable of reproducing the affection on unripe fruits only when introduced through needle-prick wounds after the milky flow from the punctured skin had ceased and had been cleared away; it was proved to be incapable of penetrating the skin of even over-ripe fruits. It is therefore possible that infection takes place through the flowers or, at least, at an early stage of fruit development, but it is also possible that the *Colletotrichum* is introduced into the older papaw fruit by a sucking insect. If infection took place through the flowers, the fungus might be found in the fruit stalk, but it has been detected in that position only once, and it may have attained it then by external accidental means. The *Glomerella cingulata* stage of the fruit strain has been obtained in culture.

13. *Vanilla*. *C. coffeanum* has been isolated from diseased areas of vanilla leaves and from a flower bud that refused to open in a normal manner, became brown in colour, and finally dropped off the plant. On the leaves, the fungus is associated with sunken grey spots which are often edged with a narrow band of dark tissue and which in time become "shot-holes"; it is accompanied at times by a species of *Fusarium* and by saprophytic forms. Two isolations of the leaf-spot *Colletotrichum* have been tested on vanilla leaves and have been found unable

\* Rev. Appd. Mycol. 1 (1922), 432.

† Loc. cit. 264.



to penetrate the unbroken skin or to enter into and live upon the leaf tissue even when this is wounded by needle-pricks, while the *Colletotrichum* isolated from the flower bud has not been shown to affect flower development in the few experiments attempted. The soft rot of vanilla ascribed to a *Colletotrichum* in Ceylon\* is not known in Uganda; indeed, the local troubles of vanilla are severely restricted, and the writer is of opinion that they may be primarily due to insect attacks.

14. *Bananas, figs and fruits of Voacanga Thouarsii* Roem. and Schlt. *C. coffeanum* is responsible for ripe rots of these fruits. There are large numbers of banana plants in parts of Uganda, but ripe rot of the fruit is remarkably uncommon and green fruit has never been found to be attacked. More tests have been made with bananas than with the other two fruits, but it seems that, in all three cases, *C. coffeanum* is unable to penetrate the unbroken skin of sound fruits. The fungus has not been found on any other parts of the respective plants. *Glomerella cingulata* has been obtained in fig and *Voacanga* cultures.

15. *Oil-palm*. The pink spore-pustules of *C. coffeanum* can often be found on the husks of ripe seeds of *Elaeis guineensis* Jacq. in the company of *Botryodiplodia Theobromae* Pat. and species of *Fusarium* and *Pestalozzia*. The fungi are confined to the outer fibrous part of the husk of the fruit, and the inner harder husk and kernel are unaffected. The harm done by any or by all of these fungi might presumably be of account in the extraction of oil from the pericarp, but it is not known how far they are responsible for deterioration of the husk. There is no local oil-palm industry of importance, and no experimental work on the tree or its products has been done. It is probable that the nut fungi are present only as ripe-rot forms and that they could be avoided by an early gathering of the fruit.

16. *Native raspberries*. *C. coffeanum* has been found on native raspberries in association with *Cladosporium herbarum* Lk. The parts played by the respective fungi have not been determined, but it was noted that the result of their attack was a complete rot of the fruit pulp.

17. *Ground-nut leaves*. *C. coffeanum* has been isolated from dead discoloured areas of ground-nut leaves. The fungus, however, was shown to be unable to reproduce the leaf spots, and, further, it is likely that the spotting was associated with a kind of rosette disease which may be shown to be due to an aphid. This strain developed perithecia of *Glomerella cingulata* on potato plugs in Roux tubes in five weeks.

\* Year Book, Dept. of Agric. (1924), 52.

MORPHOLOGICAL NOTES.

Considered apart from the question of the comparative susceptibility of the host plants, the above occurrences of *C. coffeanum* can be divided into three physiological groups, the apparently parasitic, the weakly parasitic capable of initiating ripe rots or requiring a wound entrance to host tissue, and the apparently saprophytic. The possibility of the existence of morphological features which might be distinctive of the physiological character of any particular strain of the fungus has always been kept in mind, but, on the whole, the natural morphology of *C. coffeanum* has been found to be very constant. Slight variations, however, in the colour of the pustules of conidia and in the sizes of the spores occur at times. For example, the pustules of bean anthracnose may be of a yellow tint and those of the brown blight of *robusta* coffee leaves may be almost dark grey instead of the normal pink or flesh colour. These differences of colour seem to be due partly to the substratum and partly to conditions of moisture, and they are not accompanied by differences in conidial sizes or by the presence or absence of setae, and so on. Again, when the fungus is induced to fruit *in vitro*, the conidia occasionally exceed the normal size. Thus, conidia from the fungus on discoloured cotton bolls have measured as much as  $25 \times 6\mu$ , and conidia from a diseased vanilla flower bud have appeared to be of two types when young, the first long and narrow and measuring  $22.5 \times 5\mu$  and the second shorter and broader and measuring  $15 \times 7\mu$ . Such abnormal conidia are formed only under conditions of excessive moisture, and they are not uncommon. Moreover, they are accompanied and succeeded by conidia of average size, and they give rise to normal growths of the fungus in artificial culture. The average size of conidia developed in the laboratory is  $15-20 \times 4-5\mu$ , a measurement which exceeds the figures given by Noack but which is constant in, and typical of, all the strains of *C. coffeanum*.

All the strains listed above have been grown in pure culture at one time or another, but the following notes refer particularly to recent studies of strains of *C. coffeanum* isolated from brown blight of *arabica* coffee leaves and of berries, from brown blight of *robusta* coffee leaves and of berries, from a healthy *robusta* twig after damp-chamber treatment, from brown blights of tea and orange leaves, from an orange twig, from the discoloured skin of a cotton boll, from cotton seedling anthracnose, from anthracnose of French beans, from mango ripe rot, from discoloured papaw fruit, from a diseased vanilla flower bud and from dead parts of ground-nut leaves. These strains of *C. coffeanum*

have been grown upon potato plugs in Roux tubes and on Dox, Lima bean, potato, malt extract, prune and maize-meal agars. Aerial mycelium develops in culture in two prominent ways. In one type of growth, it is large in amount and at first white in colour. Its texture is cottony or fluffy, and it covers the whole surface of the slant in a tube culture. It becomes black in contact with glass, and the white colour may persist or become dirty-white, or even greyish. In the other, it is small in amount, white or dirty-white in colour, sometimes becoming grey or black, and it is powdery in texture. In both cases pustules of conidia develop rapidly and in great numbers. In the first type the pustules may be masked by the mycelium, to which they may give a pink tinge; in the second, their presence is more apparent. At times, the pink masses of conidia may cover the whole slant and give it an oily appearance. These types of development in culture are more frequent than others, and they are regarded as typical of *C. coffeanum*. Most frequently both types appear in different cultures of all the strains, and while it seems that certain media, for example, Dox agar, tend to induce the formation of a large aerial mycelium, and others, for example, Lima bean and potato agars, tend to produce a powdery aerial growth, it can be shown that they do not do so with regularity and that either type of aerial growth may appear on any of the media. Certain strains, however, like those from mango ripe rot and vanilla flower bud, have shown a greater tendency than the others towards the smaller aerial mycelium. It has also been noted that the larger aerial growth is associated with a smaller number of conidial pustules and with a tendency to bear conidia directly upon the hyphae. Similarly, the larger aerial mycelium is always associated with the development of dark hyphae which become closely knit together to form a black leathery stroma upon, or just under, the surface of the medium and especially in the part of it below the slant when the medium of an old culture retires from contact with the glass of the tube. Stroma-formation in cultures with small aerial mycelium is less perfect and either later in appearance, or, in the majority of cases, altogether absent; it thus varies with the tendency of the strain to form a large or a small mycelium. In culture, stroma-formation follows the production of spores. Small fragments of stromatic tissue may simulate sclerotia, but true sclerotial formation has not been encountered. The pseudo-sclerotia are merely masses of hyphae, and they give rise to normal growths in culture. A dark-grey mycelium of the same type as the large white mycelium mentioned above appears at times. It develops the same black stroma, and it is associated with a large production of conidia. At other times,

the mycelium is entirely confined to the medium, the surface of which may then be, and often is, covered with pustules. Burger\*, working with strains of *C. gloeosporioides*, was able to classify them into five morphological groups according to the variations exhibited in culture, but, so far as culture studies of *C. coffeanum* have been pursued, it is impossible to adopt a similar arrangement. Certain strains resemble each other strongly, but they do not continue to do so consistently, either on the same medium or on different media, and whether of the same generation or not. The types of growth are scattered, as it were, over all the strains, and they are therefore to be taken as typical of the species rather than of individual strains. In other words, differentiation of the various strains on morphological features which are repeated with regularity is not feasible.

In young cultures, the most common or normal conidial measurements are  $15 \times 5 \mu$ . As the age of the cultures increases, conidial sizes extend to  $12.5-20 \times 4-5 \mu$  with a limit of  $25 \times 7.5 \mu$ . Such out-sized conidia as the last have appeared in the majority of the strains and on various media, and they must therefore be regarded as characteristic of *C. coffeanum* in culture rather than as the outcome of temporarily favourable conditions. They are, however, always few in number; when sown, they give growths with normal spores. The normal conidium is rounded at both ends, although the attachment-end is usually slightly narrower than the free end, but occasional crops of conidia which are pointed at one end and rounded at the other have been encountered. They have occurred in the *arabica* coffee leaf brown blight, papaw fruit, cotton seedling anthracnose and cotton boll strains, and, since they have been confined to potato media, their shape is in all probability due to conditions of growth. Similarly, irregularly shaped conidia have been found only on potato agar. The pink or flesh-coloured pustules of conidia vary in size, for a pustule may be derived from one acervulus or from the coalescence of the products of several acervuli. The pustules are usually persistent and are characteristic in varying degree of all the strains of *C. coffeanum*. They have not diminished in quantity in successive generations of the majority of the strains when grown on different media, but several of the strains have been found to relapse into infertility when grown continuously on the same medium. It should be noted, however, that none of the strains has been taken beyond the fourth or fifth generation. Non-fertile strains have been brought back to a conidial producing condition by transfers to sterilised beans, boiled rice or plugs of sweet potato. This

\* Loc. cit. 725.

behaviour seems to imply a certain morphological instability in the strains, but it is to be noted that the supposed instability is the result of an unfavourable environment and that it disappears when the environment is modified.

The appressoria are dark brown in colour, and the majority of them are simple, spherical or roughly pyriform in shape, and about  $6-7\mu$  in diameter. When they are of irregular shape, they may be  $12.5\mu$  in length. The terminal cell of the hypha or germ-tube bearing an appressorium is often swollen, and it may darken in colour and so form with the original appressorium a two-celled structure. At other times, the simple appressorium enlarges, assumes an irregular shape, develops septa, and so becomes a compound appressorium. Examination of the contents of the drop of nutrient medium used in inoculation experiments, whether successful or not, shows that the appressoria formed on germination of the conidia of the inoculum are indistinguishable from those formed in solid media or in hanging-drop cultures.

Various occurrences of *Glomerella cingulata* have already been noted. Certain strains have never yielded the perithecial form, but, in spite of this, all the strains are regarded as conidial forms of the one Ascomycete. Young ascospores are hyaline and measure  $15-20 \times 4.5-6\mu$ . When older, they become pale brown in colour and one-septate, and they may then measure up to  $25\mu$  in length. The perithecia vary in length and breadth, but the majority are about  $300\mu$  long.

A general survey of the above cultural characteristics points to the majority being the result of qualities or tendencies inherent in the various strains. Certain of them may have to be brought to light by mechanical means, but mostly they are constant enough to be raised to the rank of characters of the species *C. coffeanum*. It is to be noted that such variations as do occur are not marked enough either to enable special morphological forms to be set up or to lead to an arrangement which may be correlated with a physiological classification of the strains. A careful search has not revealed up to the present the occurrence of a saltation in *C. coffeanum*, and there has been no evidence of the splitting of a strain into morphological substrains. It appears that the morphological facies of the strains are comparatively well-marked and confined within definite limits which must therefore be taken to connote the species *C. coffeanum*.

#### CROSS-INOCULATIONS AND DISCUSSION OF RESULTS.

The above-recorded study of the morphology of *C. coffeanum* showed clearly that it was necessary to seek for a non-morphological basis of distinction between the strains, and it was hoped

that an investigation into their physiological behaviour would throw such light upon their constitution that they might be placed in well-defined groups and that some of them at least might be labelled as forms of *C. coffeanum* after the manner of the races within certain well-known species of rusts.

Throughout the inoculation experiments care was taken that the conidia from the various strains were viable when used as inocula. They were tested in hanging-drops of the same medium as was used to convey the inoculum into contact with the intended host, viz. dilute Richards' solution and sterile weak cane-sugar solution. Distilled water was not used because the nutrient solution proved more provocative of rapid spore-germination. Young freshly-produced spores of early generations were found to give the most vigorous and rapid germination and to form appressoria in the largest numbers, and they were employed in small quantities in small infection-drops. The comparative amount of germination in hanging-drop tests did not seem to be affected by the number of spores in the drop; in other words, growth was as vigorous in a crowded drop as in one with few conidia. It was also noted that germination and the formation of appressoria were frequently as vigorous in a strain of supposed saprophytic origin as in a parasitic strain. The rôle of the appressorium in infection has been shown to be a mechanical one\*, and it is presumed that the appressorium of *C. coffeanum* does not differ in function from its kind. If the formation of appressoria by a strain is assumed to indicate potential parasitism in that strain in the sense that it possesses the means of forcing an entrance into the tissues of a host, it follows that the ultimate responsibility for parasitism ought to be laid upon the host plant and its powers of resistance to attack or upon the conditions under which the fungus comes into contact with its host rather than upon the fungus itself. The act of parasitism consists of two parts, the first being actual penetration and the second the establishment and development of the parasite within its host: it is thus possible that an immune or resistant host may beat off the attack of a parasite at one or other of the stages. It has been found in these experiments that the advance of the fungus from the infection-drop into the host may be arrested at either of two points; the appressoria may fail to penetrate the host cuticle, or they may penetrate and give rise to infection hyphae which presumably meet with such a barrier to their advance that they die off and fail to establish the fungus within the tissues. In the first case, the host cuticle at the point of contact with the infection-drop is unaltered; in the second, it may be marked by a red-brown or

\* V. H. Blackman, Brit. Assocn. Report, 1924, Sect. K, Pres. Address.

brown discoloured area from which the fungus cannot be recovered and in which its presence has not been detected. It may be added that appressoria taken from the infection-drops of unsuccessful inoculations can be germinated, a fact which points to the inhibition of penetration being due to an influence outside the appressoria themselves. Unsuccessful inoculations are therefore characterised by the failure of the fungus to penetrate and establish itself in the host tissue despite the presence of viable appressoria in the infection-drop or the initial penetration by the appressoria, and the above supposition regarding the responsibility for parasitism seems to be supported by these facts. In other words, a strain may be assumed to be potentially parasitic so long as the germination of its conidia gives rise to the formation of appressoria. Experimental results, however, do not agree *in toto* with this hypothesis, which is evidently too simple to fit the case. The strains used in these experiments may be equipped with similar appressoria and they may be brought into contact with susceptible hosts under similar circumstances, but it does not follow that they will of necessity react alike. Each strain must therefore be credited with the possession of a distinct physiological character or characters which are apparently independent of such a morphological character as the presence of an appressorium. It is impossible, therefore, to guarantee to each strain in any given test the internal and external conditions absolutely necessary to its parasitism, and it is, on that account, the more essential to ensure to the strain every opportunity of doing, so to speak, its best. Care was therefore taken to use only the best available inoculum in the experiments, that is, conidia from young and vigorous growths of the first and second generations, and to provide the fungus with conditions favourable to its attack. In this way, at least two sources of variation in results were brought within control, as far as could be. It was also a regular practice in the experiments to attempt to give each strain equal opportunities of infecting the various hosts by setting up the same number of infection-drops of each, by endeavouring to ensure to each infection-drop a similar number of viable conidia through the preliminary use of hanging-drops, and by choosing for inoculation leaves and other parts of the same ages. In the cases of coffee leaves, for example, young leaves are probably less susceptible to *C. coffeanum* than older leaves, and only the latter therefore were used. It should be added that in leaf inoculations it was possible to test two and often more than two strains on one plant, a method which aided in the control of the conditions of infection. In fact, as many as six strains have been tested on a single plant, and, with large leaves like those of vanilla or *robusta* coffee, two

strains have been tested frequently on a single leaf. Again, there is no doubt that host plants, even of the same species, vary to a great extent in their resistance to an invading fungus, but no conspicuous example of such variation within a single species was encountered among the plants of these experiments. If it happened that a young plant of, say, *arabica* coffee appeared to be immune or resistant to certain strains tested upon its leaves, a further test with a strain that had been proved to cause leaf blotch in other *arabica* plants always showed that the immunity or resistance was only temporary or confined to the reaction of the plant to certain strains. The only examples of strongly resistant host species were those of the vanilla leaves and unwounded cotton bolls used in the experiments, not one of which was infected by any of the strains tested upon it, although the experiments were repeated three times. Similar cotton bolls, it may be mentioned, were infected easily by a strain of *Colletotrichum Gossypii* from Trinidad. A result was regarded as positive or, in other words, an inoculation was successful, when appropriate and typical symptoms appeared and when *C. coffeanum* was duly recovered from the material. All the experimental plants were grown in tins, and all were kept in light shade out of doors except at the time of actual inoculation and for a space of forty-eight hours immediately after, during which period the plants were covered with bell-jars. The incubation period was the same in all experiments, and it was considered to be of sufficient length because no conidia were used unless they showed a twenty to twenty-five per cent. germination in twenty-four hours. When detached parts like cotton bolls, coffee berries and fruits were inoculated, the cut ends of the stalks were sealed with beeswax, and the material was allowed an incubation period in a moist atmosphere in glass dishes and was afterwards kept under cover. All leaf and bean-pod inoculations were made on growing plants, and inoculated berries and fruits that turned brown and lost their general freshness more quickly than the controls were discarded. In order to ensure that the fungus was not aided by a previous killing of the plant surface by chemical or physical action on the part of the medium in which the conidia were suspended, drops of the media used were placed as controls alongside the inocula, and, in the few cases where the medium appeared to cause the death or degeneration of the underlying areas of plant tissue, the results were disregarded. Care was taken also to see that inoculated surfaces were wetted by the infection-drops.

It has been doubted if infection experiments of the nature of those related here are of much value in establishing patho-





genicity in, or relationship between, strains and species. Their value must vary with the conditions under which the experiments are conducted, and it follows that it is increased when the variable factors in fungus, host, and conditions of infection are brought under the greatest possible control. No doubt the control can be made more complete, but the experimental conditions described above are sufficiently standardised and simplified to enable the end in view to be attained and the results to be taken as genuine expressions of the physiological nature of the tested strains under known conditions *at a given moment*. This time reservation was originally enforced by the fact that the primary aim of investigation into the occurrence of such a species as is discussed here is usually a practical one and that, for the immediate end in view, it is necessary to consider first and foremost the nature of a strain as it is found *in situ*, that is, in infection tests carried out without delay—but additional reasons for its mention are given later. The scope of the work indicated in the table does not extend beyond those limits except in so far as it includes an effort to determine by cross-inoculations the reactions of the strains towards other hosts than the original ones with a view to their physiological classification; the results and conclusions are not presumed to have other than a local application. The Table gives the results of the inoculation and cross-inoculation experiments. A + sign denotes a positive result, and the nature of the affection caused is indicated at the top of each host column. A — sign means that the host in question was not infected by the strain in question, and a blank space means that no experiment was conducted or that the result was doubtful and was not considered of value. The symbols P, WP and S are used only conventionally and as a convenience to refer to the apparent nature of each strain at its isolation, and mean respectively parasitic, weakly parasitic and saprophytic.

The results of the direct inoculations have been mentioned in the paragraphs dealing with the hosts of *C. coffeanum* and are not all included in the Table. When the results of the cross-inoculations come to be considered, the first line of enquiry is perhaps that directed towards showing whether the experiments bring to light the existence of well-marked biological races in *C. coffeanum*. Several of the strains, particularly those marked P, have such a distinctive origin that they might be expected to behave in a distinctive manner, but, when the inoculation results are considered, many are seen to be unexpected. For example, the *arabica* coffee leaf brown blight strain of *C. coffeanum* shows a distinct partiality to coffee substrata, but it is also strictly parasitic on Pará leaves and bean pods and it causes a

mango, chilli and avocado pear ripe rot by penetrating the skins of the fruits, and a papaw ripe rot with the help of a wound entrance. At the same time, it is incapable of invading *arabica* berries through the unbroken skin, and it is partial to the leaves and wounded berries of another species of coffee, viz. *robusta*. The behaviour of the *arabica* berry strain differs from that of the leaf strain inasmuch as it is parasitic on orange leaves and non-parasitic on bean pods and Pará leaves and it can penetrate both the skin of a ripe papaw fruit and a ripe *arabica* berry without help. Similarly, the *robusta* coffee leaf and berry strains do not behave in the same fashion, for either is parasitic when the other gives negative results. On the whole, the *robusta* berry strain is more aggressive than the *robusta* leaf strain, and, in that respect, it resembles the *arabica* leaf strain more than the *arabica* berry strain. Again, the behaviour of the *robusta* coffee leaf and berry strains towards *arabica* coffee leaves is more distinctive than that of the *arabica* strains towards *robusta* leaves, but, on the whole, the results of the experiments with the four presumably parasitic coffee strains vary to such an extent that the distinctive physiological behaviour that may be expected from strains drawn from closely related sources is wanting in them whether they are considered individually or in the mass. The tea leaf brown blight strain shows distinctive behaviour in being the only strain to infect the leaves of its own host, and then only in upper-surface inoculations, but, at the same time, it is able to penetrate the uninjured skin of ripe *arabica* coffee berries and ripe mango and avocado fruits and to blight Pará leaves. It can live also upon cotton seeds and ripe *robusta* coffee berries. The orange leaf strain behaves in a manner similar to the tea leaf strain with regard to its own and the other hosts except *arabica* coffee berries and cotton seeds. The strain derived from cotton seedling anthracnose can reproduce the anthracnose through pricked seeds, can live upon *arabica* coffee berries and can cause, curiously enough, anthracnose of French beans. The bean anthracnose strain, while unable to infect coffee leaves and berries of either kind, can grow upon cotton seeds and papaw fruit, can penetrate the unbroken skin of ripe chilli and avocado fruits and can blight Pará leaves. Its physiological behaviour is therefore not very distinctive. The two WP strains from mango and papaw fruit ripe rots differ to a considerable extent in their behaviour. The former can attack bean pods and both wounded and unwounded *robusta* berries and the latter Pará and orange leaves, unripe mango fruit, ripe *arabica* berries and pricked cotton seeds and *robusta* berries, while both can parasitise *robusta* coffee leaves. A good example of a so-called S strain is that derived from

healthy *robusta* coffee material. When tested, it can infect both *arabica* and *robusta* coffee leaves, can invade bean pods and ripe avocado fruits, and can live upon coffee berries, cotton seeds and papaw fruit with the help of wound entrances. A second S strain, that from the discoloured skin of a cotton boll, can attack *arabica* coffee, orange and Pará leaves and unripe mango fruit, can penetrate the skin of ripe mango and avocado fruit and can grow upon wounded *robusta* coffee berries and cotton seed. The origin of the vanilla flower bud strain is doubtfully S, and the strain itself is certainly parasitic on bean pods, Pará leaves and ripe avocado and mango fruits. It can also penetrate into wounded cotton seed, papaw fruits and ripe *robusta* coffee berries. The ground-nut leaf strain behaves in a manner consistent with its supposed nature even when it penetrates wounded ripe *arabica* and *robusta* berries, but its ability to cause brown blight on unwounded *arabica* berries, albeit ripe fruits, shows that it can behave as a parasite on occasion. The remaining S strain is, like the ground-nut leaf strain, truly parasitic on ripe *arabica* berries. It also penetrates wounded *arabica* and *robusta* berries, and it can cause ripe rots of unwounded mango and avocado fruits.

If biological races are characterised by their obligate parasitism on certain hosts and their refusal to infect other hosts, it is apparent from the results of the above cross-inoculation experiments that none of the strains studied deserves to be set up as a distinct race. The coffee strains, for example, are not even obligate parasites on their own hosts, and they do not behave towards other hosts as a well-defined physiological group. If, again, the standard of behaviour expected from a biological race be lowered to permit of an obligate parasitism on one host and a weaker parasitism on others, the tea leaf strain alone comes near to fulfilling those conditions, but even it cannot be labelled as a truly selective strain because there is no proof that it is really more strongly parasitic on its own host than on the others it can attack. If it be admitted for the moment that educability of fungi is possible, then the most that can be said of it is that the particular strain used in the experiments has progressed further towards acquiring the habit of selective parasitism than any of the others. On the same condition, it may be added that, as far as the results of the experiments may be taken to indicate the physiological natures of the tested strains, none of them has begun to acquire the power to penetrate green cotton bolls and so become capable of causing boll anthracnose, and that, on the other hand, a large proportion of them is able to attack bean pods, a substratum which may be expected to require a specialised race for its penetration.

There is, on the whole, a remarkable lack of distinctive behaviour among the strains, and it is impossible to apply to any of them such a test of exact relationship as can be applied with success to strains of, for example, *Puccinia graminis*, a test in which infection by a given strain implies that the host infected must be a certain pure-line wheat and no other.

It is evident that certain of the host plants or parts of plants used in the experiments are more susceptible to penetration by *C. coffeanum* than others. Among the most susceptible are Pará leaves and ripe avocado and mango fruits. French bean pods are surprisingly susceptible, and *robusta* coffee leaves are more susceptible than *arabica* leaves. In these cases, however, emphasis may be laid on the necessity for penetration before infection can take place, and it follows that a strain may be regarded as truly parasitic at the moment it is able to penetrate any one or more of these host parts. The susceptibility factors of the different hosts may be left temporarily out of account when all the strains are tested on each of these hosts under similar conditions, and the results of such one-host tests may be regarded as an index to the character of each strain. The behaviour of the strains may thus be examined from the point of view of the physiological origin of each (denoted by the symbols P, WP and S) with a view to showing whether they retain in a marked manner their supposed original character. With regard to the six susceptible hosts or parts mentioned above, the *arabica* coffee leaf strain alone can parasitise all of them, while the remaining three coffee strains infect either three or four of them with a certain amount of unanimity. The behaviour of the other P strains varies between the three similar infections by the tea leaf and orange leaf strains, the single bean pod infection by the cotton seedling anthracnose strain, and the three infections of the bean anthracnose strain. The two so-called WP strains each parasitise three of the six hosts, two out of the three being alike in both cases. The behaviour of the supposed saprophytic strains is perhaps more interesting than that of the parasitic strains inasmuch as those from the healthy *robusta* coffee twig, the discoloured cotton boll and the vanilla flower bud can behave in a truly parasitic manner, although ripe avocado fruits are the only host common to all three strains. The orange twig strain can penetrate ripe avocado and mango fruits, and the ground-nut leaf strain is non-parasitic in all six tests. When the behaviour of the strains is regarded from what may be termed the weakly-parasitic point of view, that is, towards such hosts as wounded *arabica* and *robusta* coffee berries, cotton seeds and papaw fruit, it is seen that only the *arabica* coffee leaf and the tea leaf strains out of the eight

supposed P strains infect all four hosts, and that the four coffee strains do not behave in a similar manner. The P strains are on the whole less strongly parasitic in these cases than in those where penetration of the host is a preliminary to parasitism. The WP strains may be expected to grow upon all four substrata, but they do not do so. On the other hand, one of the S strains, that from the *robusta* coffee twig, grows upon all the substrata, while the others are parasitic in a degree equal to that of certain of the P strains. If the conditions on which the strains are judged are modified to include more rigorous tests of parasitism, viz. the ability to penetrate what appear to be the least susceptible hosts, unripe mango fruit and unwounded ripe chilli pods, it is found that the only strain which is successful in both tests is the P strain from *robusta* coffee berry brown blight. Two other parasitic strains, those from *arabica* coffee leaf brown blight and bean anthracnose, are successful in one test only, while the remaining so-called P strains do not penetrate either substratum. One of the WP strains is parasitic on the green mango fruit, and only one of the five S strains succeeds in attacking one of the two hosts, the unripe mango. The results of these last tests show that the parasitic strains are successful in one test out of four and that the saprophytic strains are successful in only one test out of ten, and may be taken to prove that an originally parasitic or saprophytic strain tends to retain its parasitic or saprophytic nature. Such a deduction, however, is not supported by a similar analysis of the results of the tests on the six hosts which are supposed to be more susceptible than the green mangoes and chilli pods or of the tests on wounded host parts. In any case, the supposedly more rigorous tests are hardly numerous enough to justify deductions from them. It should be added that, for reasons which are indicated later, the results do not lend themselves to statistical treatment, and that it is realised that the grounds on which a strain is labelled P, WP or S are open to criticism. It can only be concluded that there is no proof that the original nature of a strain has any influence on its subsequent behaviour, and it follows that the attempt to denote the nature of each strain at its isolation by a label which is intended to indicate a permanent quality has no scientific basis.

It might have been of interest to enquire whether a group of related strains like the four derived from coffee leaves and berries shows a restricted or limited choice of host substrata, but it is evident that no stress can be put upon the behaviour of strains which do not behave consistently towards their own hosts. All that may be said of them is that they have an affinity for ripe mango and avocado fruit, and even that statement has little

value since these fruits may be suspected of being susceptible to *C. coffeanum*. There is a general lack of direction of attack among all the tested strains, a state of affairs which is perhaps a natural accompaniment of the lack of physiological specialisation.

It would be easy to close the discussion at this point by concluding that *Colletotrichum coffeanum* is, under the conditions related, a composite species made up of numerous units which are not distinguishable from each other on either morphological or physiological grounds, but, unfortunately, that statement does not really explain the nature of the fungus which, after all, it was the object of this investigation to disclose. The fungus may be regarded as a plastic organism of which the morphological aspect is relatively constant while its physiological aspect varies with changes in the environment, and it may be assumed that its parasitism is conditioned by the presence of the fungus among hosts which are more or less susceptible to its attacks under environmental conditions which are more or less favourable to either parasite or host. If this interpretation of its nature is taken to mean that *C. coffeanum* is always lying in wait, as it were, for opportunities of attack that are granted or denied to it by temporarily favourable or unfavourable conditions, it may be taken also to imply that the strains of the fungus are always potentially parasitic both in themselves, that is, each towards its own host, and also towards other hosts. In other words, it may be assumed that each strain of *C. coffeanum* is not only a stable morphological unit but also a discrete physiological entity which can and ought to behave in a certain definite manner under certain known environmental conditions. That it does not do so is proved by the mixed and almost erratic results of the cross-inoculations which, it may be emphasised, were carried out under controlled and constant conditions favourable to the fungus, by the existence in nature of apparently saprophytic strains, and by the fact that in no case has any one strain which yields a + result been found capable of one hundred per cent. success in causing infection. A + result implies only that a given strain has infected a given host at least once out of the number of opportunities afforded it, and it does not infer any definite percentage of infection. Further, it has been found that the percentages of + results from any given strain diminish with the age of the strain in culture and with the use of infection conidia drawn from generations beyond the first and second. For example, tests of the *robusta* coffee leaf brown blight strain on its own host give + results which vary from a fifty per cent. infection with conidia from a young culture (seven days or less of age) to a nought per

cent. infection with conidia from a culture four weeks old, and, in certain cases, no infection at all has been got with conidia from the fourth or fifth generation of a strain which behaved in a different manner in its first and second generations. An alteration in the conditions of experiment favourable to the fungus, for example, a lengthening of the incubation period under the bell-jar, does not affect these results. The results of a given set of experiments cannot be expected to repeat themselves unless the inoculations are carried out with material from young cultures of an early generation, and, in any case, it has been found that different isolations of a given strain cannot be guaranteed to behave in an exactly similar manner. There were, therefore, extra reasons in the experiments for using conidia from what have been called young and vigorous growths and also for the mention of a time reservation in the account of the conditions of experiment.

Certain of the above facts may be expressed in a different manner by saying that the strains of *C. coffeanum* lose with age their capacity for parasitism, and on the whole they seem to imply that the strains in question are genetically unstable. It has already been shown that the strains do not behave as physiological entities. As for their supposed instability, it can be said that there is a steadily accumulating body of evidence in favour of the view that genetic stability is as constant a character of fungi as of higher plants. If that view be accepted, it is necessary to premise the existence of discrete physiological strains capable of different types of behaviour within any single isolation of *C. coffeanum*. No strain is to be regarded, then, as a simple physiological entity, even at its point of origin, and each may be what Brierley has called a "genetic population."\* No immediate proof of the presence of more than one physiological strain within a morphological unit of *C. coffeanum* can yet be advanced, but the writer hopes to continue the study of the fungus. In the meantime, the "genetic population" explanation fits the facts and throws light upon the curious behaviour of the strains of the fungus under discussion. It also explains the state of affairs in the field where plant disease due to *C. coffeanum* ought to be more in evidence than it actually is. Hosts and fungus have a common geographical distribution, and natural conditions, particularly cool dewy nights and misty mornings, are favourable to fungus attack. No doubt, also, certain of the substrata on which *C. coffeanum* is found are susceptible to its advances and open to its attack. The fungus may therefore be supposed to consist of numerous physiological strains of different natures living side

\* Report of the Imper. Bot. Conference, London, 1924, p. 117.



by side, certain of which from time to time find suitable opportunities of adopting the kind of life which lies within their physiological capabilities, and it follows that the limiting factors governing local *C. coffeanum* disease are susceptibility of the host part exposed to attack and the presence of a strain possessing certain germinal qualities.

#### CONCLUSION.

It was made clear at the beginning of these notes that the species name *C. coffeanum* was adopted only for reasons of local convenience, and the fact that the forms which come under the scope of the present inquiry can be included under the one name on morphological grounds has been emphasised. The majority of them, if not all, are regarded as conidial forms of *Glomerella cingulata*, and relationship with that Ascomycete has been proved for many of them. It was also mentioned that specific names other than *C. coffeanum* might be used with equal or greater justification to designate the various forms or all of them, and it follows that the study of isolated occurrences of the fungus with special reference to the host of each instead of in the mass would lead to the use of the other specific names. Moreover, both the generic names *Colletotrichum* and *Gloeosporium* would have to be used. In such circumstances, the fungus on coffee leaves and berries might be called *C. incarnatum* Zimm., that on cacao *C. incarnatum* or *C. theobromicolum* Del., that on tea *C. Camelliae* Mass., that on citrus, mango and avocado pear *C. gloeosporioides* (Penz.) Sacc., that on chillies *C. piperatum* Ell. and Everh. or *C. nigrum* Ell. and Hals., and that on papaw and figs *C. Caricae* Stev. and Hall. The foregoing list of names might be extended to include others, but it is confined meantime to those species whose connection with *Glomerella cingulata* has been proved either here or elsewhere. Among other species that may come into question are *C. Heveae* Petch, *C. Musarum* Cke. and Mass. and *C. Lindemuthianum* Br. and Cav. *C. Gossypii* Southw. is definitely excluded because the Trinidad strain already referred to was found to differ consistently in culture from *C. coffeanum* in the dry powdery appearance it imparted to the surface of the medium. The position of *C. Lindemuthianum*, again, is less clearly defined than that of *C. Gossypii*. In both the occurrences on bean plants mentioned under a previous heading, the local fungus was indistinguishable from *C. coffeanum* in nature and in culture, and, in one of them, that in which the fungus was associated saprophytically with a *Sclerotium bataticola* wilt of beans, an isolation of it was identified by the Imperial Bureau of Mycology as *C. Lindemuthianum*. The same

form produced a *Glomerella* stage which was indistinguishable from that of other *C. coffeanum* forms. The bean pod anthracnose strain, however, has not formed the perfect stage and has not been compared with material from other sources, and *C. Lindemuthianum* therefore is in the meantime excluded from the list. It may be added that, in the separate descriptions of the species of the above list, the morphological details which vary from the details of *C. coffeanum*, for example, sizes of acervuli and conidia, colour of conidial masses, presence or absence and sizes of setae and also types of appressoria, are repeated and paralleled here and there in the occurrences of *C. coffeanum* both in nature and culture. It is clear that the local use of the above names for conidial forms which resemble each other strongly and which behave in the manner of *C. coffeanum* is to be deprecated, and the writer is of opinion that the best method of naming them is to apply the conidial name which has priority among those which have a proved connection with *Glomerella cingulata*, namely, *C. gloeosporioides*. Distinct biological races can then be designated as forms of the species, for example, forma *Camelliae*. The fact that no biological races have been found yet does not preclude their undiscovered existence in the present or, as some authorities would admit and others deny, the possibility of their evolution in the future.

#### SUMMARY.

1. Various occurrences of a species of *Colletotrichum* which have common morphological features in nature and culture are outlined.
2. An attempt is made to find whether the strains of the *Colletotrichum* are characterised by constant physiological qualities which lead to consistent behaviour and a definite relationship between them and their host or hosts.
3. It is found that the strains do not behave as physiological entities and that they appear to be genetically unstable. An explanation of their physiological variability is advanced, and it is suggested that they should be named in a certain way.

## BLACK RUST IN SCOTLAND.

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(With Plate IV.)

### INTRODUCTION.

#### History.

AMONG the rusts of cereals, that known as "Black Rust" has long been recognised as a disease causing the most widespread and serious loss. The name *Puccinia graminis* was given to the teleutospore stage of this fungus in 1797 by Persoon and at the same time the rust on barberry was named *Aecidium Berberidis* by the same investigator, who did not associate the two diseases. The uredospore stage was called *Trichobasis linearis* Lév.

In 1864 de Bary as a result of infection experiments, used the name *Puccinia graminis* to include all three stages of Black Rust and in 1881-2 de Bary's results were confirmed by Plowright (6, 7). Early records of the distribution of Black Rust in Scotland are given by Stevenson (10) in 1879. Trail in 1890 (11) published his *Revision of the Uredineae of Scotland* and in this he records the aecidial stage on the barberry for the Clyde, Forth, Tay, Dee, Moray and Orkney districts the other stages being found in addition in Ross but not in Orkney.

Eriksson in 1894 gave the first account of specialisation in Black Rust. Since then Eriksson (1, 2) and Henning and Stakman and Piemeisel (8, 9) have published several papers on the subject. According to these investigators there are seven biologic forms of the rust in the States; reference is also made to an Australian form of *Puccinia graminis Tritici*. These forms are as follows:

*Puccinia graminis Tritici* Erikss. & Henn. (American form).  
(Australian form).

*Puccinia "graminis Tritici compacti* Stak. & Piem.

*Puccinia graminis Secalis* Erikss. & Henn.

*Puccinia graminis Avenae* Erikss. & Henn.

*Puccinia graminis Phlei-pratensis* Stak. & Piem.

*Puccinia graminis Agrostidis* Erikss.

*Puccinia graminis poae* Erikss. & Henn.

In England Mehta, working at Cambridge<sup>(5)</sup> found Black Rust present on wheat, barley and couch. His experiments showed these infections to belong to two biologic forms—*Puccinia graminis Tritici* on wheat and *Puccinia graminis Secalis* on barley and couch.

### *Scope of the present investigation.*

Though the rust has long been known in Scotland, no effort appears previously to have been made to define its economic importance. The present investigation aims at indicating the forms of the rust which occur in Scotland.

It appears that no recent records have been made from some of the districts mentioned by Stevenson and Trail but no extensive study of the present distribution has yet been made.

In 1914 *Puccinia graminis* was observed on barberry and *Agrostis vulgaris* near Tyndrum in Argyllshire. Since then the rust has also been noted in a few other scattered localities in Scotland, namely Inverness-shire, Fifeshire, Midlothian, Berwickshire, Peeblesshire and Dumfriesshire. The aecidial host *Berberis vulgaris* is probably not so common as it once was, but is found widely distributed in a healthy condition over the southern half of Scotland.

### *Experimental methods.*

In 1924 it was decided to investigate the specialisation of the forms of Black Rust which has been found in Midlothian and Dumfriesshire. In addition to field observations, experiments were carried out at the Royal Botanic Garden, Edinburgh, in the field and laboratory.

The following results were obtained from infection experiments:

Midlothian: wheat, barley, rye\*, *Agropyron repens*\*, *Bromus sterilis* infected.

Berwickshire: oats, barley, *Agropyron repens* infected.

Dumfriesshire: oats\*, *Agropyron repens*\*, *Agrostis vulgaris*, *Dactylis glomerata* infected.

Argyllshire: *Agrostis vulgaris* infected.

*Agrostis vulgaris*, *Dactylis glomerata*, *Phleum pratense*, *Arrhenatherum avenaceum*, *Lolium perenne*, *Poa nemoralis* and *Festuca elatior* were present but not infected in Midlothian: these species and also wheat were present but not infected in Berwickshire.

The teleutospore stage on those species marked with an asterisk served as inocula. The healthy barberries for the experiments were obtained from the Royal Botanic Garden, Edinburgh. The infected Gramineae used for inocula were collected in autumn and allowed to over-winter outside the laboratory, care being taken to prevent cross contamination.

As regards the infections with the Dumfriesshire material—teleutospores on oats and couch—the following is an outline of the methods:

In out-of-door infection from rusted oat straw, this was allowed to winter under wire netting on the ground immediately beneath six healthy barberry bushes. In the spring the rusted straw was tied to the barberry branches before the buds opened. Some of these branches were enclosed in glass flasks to increase the chances of infection (Pl. IV, fig. 1). The flasks were plugged with cotton wool, and the contents were kept moist throughout the season by means of a spray.

Teleutospores from the infected straw were examined for germination in the laboratory at intervals from January till April. No germination was observed either in hanging drops or in a moist Petri dish until about the second week of April.

For infection from couch, the same methods were adopted as regards attachment of the rusted straw to the branches of six barberry bushes and the enclosure of some of these branches in glass flasks.

When teleutospore germination was found to take place in spring, laboratory experiments were undertaken. Two potted barberry bushes were infected with oat rust, and one with couch rust, by tying the rusted straw round young shoots (Pl. IV, fig. 2) and also by placing teleutospores on the moistened leaves. The plants were covered with bell-jars and kept moist by means of a spray. Healthy barberries were also infected indoors from rusted rye and couch obtained in Midlothian. The method of infection was similar to that with the Dumfriesshire material.

In the laboratory the method of infection experiments with the cereals and grasses was as follows: Grains of the cereals and roots of healthy grasses were grown in pots under bell-jars. When the barberries, artificially infected, showed ripe aecidia, these were transferred by means of a brush to the moistened leaves. Later on, in order to save space and to increase the chances of infection, the cereals and grasses to be tested for any form of the rust were placed under the same bell-jar as an infected barberry (Pl. IV, fig. 3).

Out-of-doors, grain of rye, barley and wheat were sown in an oat field immediately behind a naturally infected barberry

hedge in Midlothian near which in the previous year only couch was found rusted. Oats, *Agrostis vulgaris* and *Agropyron repens* were grown out-of-doors at the Royal Botanic Garden, Edinburgh and infected artificially with aecidiospores from artificially infected barberries. Owing to the amount of rain at the time of inoculation it was necessary to cover the plants with bell-jars.

#### RESULTS AND SPECIALISATION.

The results of inoculation experiments with aecidiospores from barberries, which had been infected from teleutospores from *Agropyron repens*, were that material from Midlothian (April 7th, 1925) and Dumfriesshire (April 8th) barley, rye and *Agropyron repens* gave positive results: wheat, oats, *Agrostis vulgaris*, *Dactylis glomerata* and *Phleum pratense* negative results.

Inoculations with aecidiospores from barberries which had been infected from teleutospores from rye, showed that with material from Midlothian (April 18th) barley, rye, *Agropyron repens*, were infected, while wheat, oats, *Agrostis vulgaris*, *Dactylis glomerata* and *Phleum pratense* were not infected.

The results of inoculations with aecidiospores from barberries which had been infected from teleutospores from oats, were that with material from Dumfriesshire (April 8th) oats and rye gave positive results; wheat, barley, *Agropyron repens*, *Agrostis vulgaris*, *Dactylis glomerata* and *Phleum pratense*.

The results show that the rust, originally from *Agropyron repens*, obtained from both Midlothian and Dumfriesshire, is capable of infecting the same hosts as the form on rye, and that the oat form of the rust infects oats and to a certain extent rye also.

The results of laboratory experiments with the rust on rye and *Agropyron repens* agree, so far as they have been carried out, with those obtained by Stakman and Piemeisel with *P. graminis Secalis*.

The form on oats, in so far as it only infected oats and, to a less extent rye, in the experiment is *P. graminis Avenae*.

Negative results were obtained in infection experiments with the above two forms on wheat, *Agrostis vulgaris*, *Phleum pratense* and *Dactylis glomerata*. *Bromus sterilis*, the only other grass found infected in the field has not been experimented with.

Barley, according to Stakman and Piemeisel, is, like rye, a host to a varying extent, for all seven recorded forms of the rust. With both the Midlothian and Dumfriesshire material of *P. graminis Secalis*, the degree of infection on barley in the field

and more especially in the laboratory would indicate that it bears this form, which is said most readily to infect it.

Specimens of infected wheat, obtained in Midlothian, evidently bear the form *P. graminis Tritici*; only one other form, *P. graminis Secalis*, is capable of attacking it and laboratory experiments with this form gave negative results.

No infection was obtained on *Agrostis vulgaris*, *Phleum pratense* and *Dactylis glomerata* with the *Secalis* or *Avenae* forms.

As regards the *Agrostis vulgaris* and *Phleum pratense*, these apparently bear two distinct forms, *P. graminis Agrostidis* and *P. graminis Phlei-pratensis* respectively. The former, so far as American experiments have shown, does not affect *Phleum pratense*, nor the latter *Agrostis vulgaris*. Both grasses have been weakly infected by artificial inoculation in the States, where also *A. vulgaris* was inoculated but not infected with *P. graminis Secalis*.

As regards the rusted *Dactylis*, this was found only in Dumfriesshire where it was growing alongside infected oats, *Agropyron repens*, *Agrostis vulgaris* and *Phleum pratense*. The *D. glomerata* growing alongside infected *A. repens* in Midlothian, and alongside infected oats and *A. repens* in Berwickshire was clean.

In the States *P. graminis Avenae* is found on *D. glomerata* in nature, but *P. graminis Secalis* was neither found on this grass in nature nor was it able to infect it after inoculation. The present infection experiments on *D. glomerata* in the laboratory gave negative results with both forms.

It is unlikely therefore that the *Dactylis* from Dumfriesshire bore either of these two forms. The only other possible forms that it could have borne are those on *Agrostis vulgaris* and *Phleum pratense*. *Dactylis* has been stated to be susceptible to *P. graminis Agrostidis* in the laboratory, but to be a host in nature for *P. graminis Phlei-pratensis*. Probably because the *Dactylis glomerata* bore the form *P. graminis Phlei-pratensis*.

So far, therefore, as observations have been made and experiments carried out, these indicate that the following five forms of *P. graminis* occur in Scotland:

- P. graminis Tritici* Erikss. & Henn.
- P. graminis Secalis* Erikss. & Henn.
- P. graminis Avenae* Erikss. & Henn.
- P. graminis Phlei-pratensis* Stak. & Piem.
- P. graminis Agrostidis* Erikss.

# ECONOMIC IMPORTANCE OF BLACK RUST AND EFFECT OF BARBERRY ERADICATION.

The destructive effect of the fungus on cereals would account for attention being drawn to it since early times. Centuries ago it was observed that a definite relationship existed between the rust on barberry and the Black Rust on cereals; also that the eradication of barberry bushes from near infected fields resulted, in temperate climates at any rate, in a disappearance of the rust on cereals.

Only within recent years, however, have any statistics been obtained on the loss attributable to *Puccinia graminis*. In the United States of America, Canada and Australia estimates indicate that the damage due to this rust alone amounts to millions of pounds yearly. In Denmark the rust was, until the recent measures for control were undertaken, a great source of loss. In England and Wales the rust is said to injure wheat seriously, causing the grain to be small and shrivelled, and even to render crops worthless. In England the spores of the fungus are said to spread the disease for many miles, and eradication of the barberry has been recommended.

In Scotland no attacks on an epidemic scale have been recorded; and until a complete survey has been made, it is not possible to state the amount of loss. Judging from the extent of the rust in the localities in which it has been recently observed, it would appear that the rust may spread to a distance of about one mile from affected barberry during the summer; but that the amount of rust diminishes rapidly at a short distance from the affected bushes.

A number of observations were made in Midlothian on the distribution of the rust and the time of infection of a number of grasses. The results are given in the Table below.

Table.

Host Plant	Distance from infected Barberry	Infection	
		July	September
<i>Agropyron repens</i>	Immediately below	+	+
"	200 yards	+	+
"	1 mile	-	+
Barley	Immediately below	+	+
"	15 yards	-	+
"	1 mile	-	-
Rye	Immediately below	+	+
"	1 mile	-	+
Wheat	Immediately below	-	+
"	1 mile	-	+



From the above Table it is evident that the barberry carried a double infection, viz. *P. graminis Secalis* and *P. graminis Tritici*.

Of the hosts infected by the former *Agropyron repens* appears to be the most susceptible and the first to be infected although even this species was not infected at a distance of one mile until September. Rye is the next in order of susceptibility and barley least susceptible as it was not affected in July even as close as fifteen yards. It would appear therefore that the stock of *P. graminis Secalis* is maintained by the barberry and the couch immediately below it and that this form spreads through the summer on the couch to the rye and barley.

In Dumfriesshire in September, 1924, oats was found to be infected up to seven hundred yards from infected barberry but observations could not be made at greater distances; the amount of infection steadily diminished with increased distance.

As early as 1660 according to Loverdo<sup>(4)</sup> an Act of Parliament was passed at Rouen condemning the planting of the barberry in the vicinity of grain fields. A similar law according to Plowright was passed in Massachusetts in 1755 with favourable results.

The justification of this legislation was shown in 1864-5 when de Bary definitely proved the relationship of the rust on these two hosts.

The best example of the effect of barberry eradication is that started in Denmark in 1903. The beneficial results of that campaign are recorded by Ferdinandsen<sup>(3)</sup> in 1924. With the almost complete eradication of the barberry in Denmark the Black Rust on cereals has practically ceased to exist. In England similar control, on a smaller scale, is yielding satisfactory results.

#### CONCLUSIONS AND SUMMARY.

Black Rust occurs in Scotland in scattered areas and only in the immediate vicinity of affected barberries is the infection on cereals and grasses heavy. There is no evidence that the disease is carried over the winter by means of uredospores and destruction of barberries will almost certainly result in the disappearance of the disease.

Observations in the field show that the following five forms of *Puccinia graminis* are present in Scotland: sp. f. *Tritici*, *Secalis*, *Avenae*, *Agrostidis*, *Phlei-pratensis*.

Infection experiments with sp. f. *Secalis*, *Avenae* and *Agrostidis* indicate that these forms have the same powers of infection as have already been described in England and North America.

The authors wish to express their indebtedness to Dr Malcolm Wilson, Mrs N. L. Alcock, and Messrs J. S. L. Waldie and



Fig. 1.

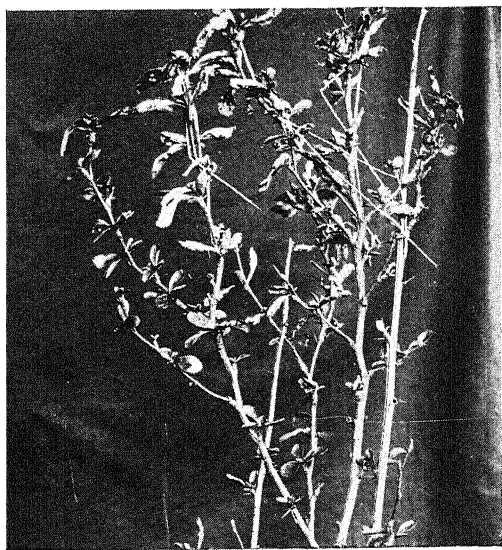


Fig. 2.

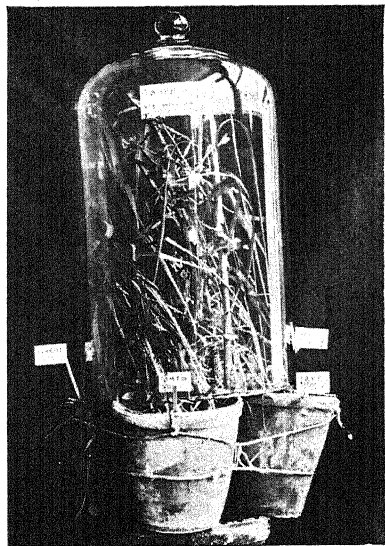


Fig. 3



J. Fraser for assistance in obtaining material and for information about rusted crops and grasses; also to Mr Harrow, Curator of the Royal Botanic Garden, Edinburgh, for the provision of barberry plants and of ground for out-of-door experiments.

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#### EXPLANATION OF PLATE IV.

- Fig. 1. Artificial infection of barberry out-of-doors. In the flask and on the ground are straws of couch bearing teleutospores of *Puccinia graminis*.
- Fig. 2. Infection of barberry indoors. Straws bearing teleutospores are tied to the branches. A bell-jar covers the plant.
- Fig. 3. Infection of cereals indoors. Four small pots in which cereals are growing are resting on a larger pot containing a barberry plant. The leaves of the cereals are thus growing among the leaves of the barberry, which bear aecidia.

## A PRELIMINARY ACCOUNT OF A DISEASE OF GREEN COFFEE BERRIES IN KENYA COLONY.

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WHAT is believed to be a previously unrecorded disease of coffee exists in Kenya Colony and has been the subject of investigation for some time. The main facts concerning its causation and control are now known but much work remains to be done in amplifying a number of points.

The disease, which is known locally as Black Berry or Coffee Berry Disease, was first reported at the end of 1922 and has

caused considerable annual losses, in some cases as much as seventy-five per cent. of the crop having been destroyed. Fortunately, it is known to occur only in two small districts, which are situated at altitudes of between six and seven thousand feet and characterised by an average annual rainfall of fifty to seventy inches, the greater part of which is spread fairly evenly over eight or nine months of the year.

#### SYMPTOMS.

The disease is confined almost entirely to the berries, berry stalks and a small area of the twigs immediately adjacent to the bases of the berry stalks. The green berries are attacked at all ages, a small dark brown spot being the first symptom. This enlarges in area, becomes slightly sunken and eventually involves the whole berry, the pulp of which becomes brown, hard and brittle. Except for the fructifications of the fungus, the surface of the dried-up berries is smooth. Examination of the coffee beans shows that the one immediately under the point of infection becomes diseased very soon after the external spot appears, and that almost as soon as the pulp is completely involved, both beans are reduced to a shapeless, black, shrivelled condition. They then occupy very little of the internal space of the berry and are completely useless commercially. The berry stalks are soon quite desiccated and black, but the fungus does not appear to be able to progress more than a few millimetres along the twig. Many of the dead berries fall to the ground. The branches, however, are not killed and their leaves remain perfectly healthy in appearance.

In the field the pulp is often found to be covered with numerous minute black fructifications from which pink spore masses, becoming white with age, are produced. Microscopic examination shows the fructifications to be the acervuli of a species of *Colletotrichum*, morphologically indistinguishable from those of *Colletotrichum coffeanum* Noack. The acervuli contain dark brown, tapering setae, with four or fewer septa which measure  $84-103\mu$  in length. The spores are typical in shape and measure  $13.4-18.5\mu$  by  $4.8-6.6\mu$  with average dimensions of  $15.1\mu$  by  $5.7\mu$ .

There is reason to believe that the Coffee Berry Disease existed in the affected districts for at least one or two seasons before it was reported and that it was mistaken for other coffee maladies. Chief of these was dieback; a condition of the bushes produced by the death of the primaries from the tips downwards. In Kenya this is almost always due to non-parasitic agencies and differs from what is seen in Coffee Berry Disease, as all the leaves and other appendages attached to the branch die with it.

Brown blight, as described by Butler<sup>(1)</sup>, in India, is widespread in Kenya but is on the whole unimportant. It usually attacks the berries in the ripe or nearly ripe stage only, and, beyond staining the bean slightly and making the pulping operation somewhat difficult, does little harm. It, however, has been confused at times with the Coffee Berry Disease. It occasionally happens that, owing to sudden ripening of the crop or other cause, it is impossible to pick the berries as fast as they ripen. If such mature coffee is left on the bushes for long, it dries up and becomes black and is then known as *buni*. This condition has also been mistaken for Coffee Berry Disease but may be distinguished from it by the fact that *buni* berries always contain fully developed beans of marketable value. Recently Thomas<sup>(2)</sup> has mentioned a disease in India, which is known as black bean or *jollu*. It causes staining, shrivelling or rotting of the coffee bean, and it is sometimes accompanied by the formation of a brown liquid. No pathogenic organism has been detected in connection with the trouble. It seems unlikely that it is in any way connected with the Kenya disease with which no wet-rot has ever been associated and no difficulty experienced in discovering a pathogenic fungus. Dr C. H. Gadd informs me that green coffee berries in Ceylon are sometimes attacked in a manner similar to that seen in Kenya. The Ceylon disease, however, has not received much attention and cultures which Dr Gadd kindly isolated for me from one such diseased berry differ markedly from those isolated in this country.

#### THE FUNGUS.

Numerous cultures on carrot agar were made from diseased pulp, beans, berry stalks and portions of twigs from three different plantations in one of the districts and from one plantation in the other. The material was invariably externally sterilised in 1/1000 mercuric chloride solution before plating, but, as the specimens had to be brought nearly two hundred and fifty miles to the laboratory, a journey often taking a week, it was not surprising to find that a mixture of fungi appeared in the cultures. In more than seventy-five per cent. of the cases, however, a fungus appeared in the cultures which was white at first, became dark green later, and produced spores of the *Colletotrichum* type. Where the cultures originated from the shrivelled beans this fungus developed pure in a large proportion of them. In addition a species of *Phoma* occurred with moderate frequency, while one or two species of *Fusarium*, a *Macrosporium* and bacteria occurred so irregularly as to appear negligible as causal agents

## INOCULATION EXPERIMENTS.

Soon after the first isolations were made, a preliminary inoculation test was carried out on some green coffee berries on an experimental farm near Nairobi. Only the *Colletotrichum* cultures were used. About five per cent. of the inoculated berries developed typical symptoms and the fungus was afterwards re-isolated from them. This percentage of successful results was not considered sufficiently conclusive evidence of the cause of the disease. The bushes used, however, were particularly vigorous and were growing under very different conditions from those in the affected districts hundreds of miles away. It was decided, therefore, to carry out further inoculations in one of those districts.

For this purpose pure cultures were prepared of the *Colletotrichum* and the *Phoma* found in connection with the disease. The latter fungus was included because it did not appear so rarely in the original isolations as to justify its omission. Culture tubes of *Colletotrichum* isolated from a brown blight spot on a coffee leaf growing at Nairobi were also prepared. These were taken, in July, 1924, to an estate in the affected district and inoculations were carried out on green berries in all stages of development in two plantations approximately a mile apart.

Inoculation was effected by inserting mycelium and spores into small punctures on the berries made by the point of a sterilised needle. Only healthy branches were utilised and in each case a neighbouring branch of the same bush was used as a control and a similar number of berries was treated in exactly the same way except for the omission of the inoculum. The inoculated branches and controls were enclosed immediately after treatment in waxed paper bags, which had small pinholes on the under sides to avoid the accumulation of excess moisture within.

The brown blight *Colletotrichum* was inoculated into seventy-five berries and the *Phoma* sp. into forty-eight. At the end of six weeks all the treated berries and controls were perfectly healthy. The *Colletotrichum* which had been originally isolated from berries affected with Coffee Berry Disease was inoculated into about one hundred and fifty berries. After five days, two berries showed a small discoloured area at the inoculation puncture and typical symptoms speedily developed. Two days later, concentrically arranged acervuli were visible on the disease spots, and numerous other inoculated berries were becoming diseased. Six weeks after inoculation, seventy-two, or about forty-eight per cent., of the inoculated berries had become

typically diseased. From these the fungus was afterwards recovered in culture. All the controls remained free from disease.

Cultures made by plating short lengths of twigs bearing the dead stalks of some of the berries successfully inoculated in the above experiment developed a growth of the Coffee Berry Disease fungus only from the first few millimetres of the twigs immediately adjoining the berry stalks.

In 1925, during a later visit to one of the affected districts, the opportunity was taken of carrying out more inoculation experiments. The particular object was to explore further the possibility that more than one strain of *Colletotrichum* from coffee might be capable of producing the same symptoms on green coffee berries. The strains used were from a coffee leaf and a coffee berry respectively, both of which were attacked by brown blight. In addition, *Colletotrichum gloeosporioides* isolated from a lemon leaf was used, and, for comparative purposes, the Coffee Berry Disease *Colletotrichum* which had been obtained from the second affected district, a hundred miles away, was inoculated into some berries at the same time. The method employed was the same as that of the previous year, and the results of this series of inoculations are summarised in Table I.

Table I. *Showing results of inoculation of green coffee berries with Colletotrichum from various sources.*

Date	Source of <i>Colletotrichum</i> used	Number of berries treated	Diseased berries when examined on		
			29. vi. 25	7. vii. 25	14. vii. 25
19. vi. 25	Control	30	Nil	Nil	Nil
	Berry affected with Coffee Berry Disease	20	6	13	13
20. vi. 25	Control	20	Nil	Nil	Nil
	Coffee leaf	40	Nil	Nil	Nil
20. vi. 25	Control	20	Nil	Nil	Nil
	Coffee berry affected with brown blight	40	Nil	1?	2
20. vi. 25	Control	20	Nil	Nil	Nil
	Lemon leaf	40	Nil	Nil	Nil

It will be seen from the Table that, apart from the actual Coffee Berry Disease fungus, the only *Colletotrichum* which produced disease in the berries was that obtained from a berry affected with brown blight. This, as has been previously explained, is regarded by the writer as a quite distinct disease. In the case of the two diseased berries, owing to difficulties which need not be gone into, no attempt was made to re-isolate the fungus. Nevertheless, even assuming that the disease was definitely caused by the brown blight *Colletotrichum*, it will be



seen that no signs were noticed until more than double the time required by the Coffee Berry Disease fungus to produce noticeable symptoms had elapsed. This applies both to the inoculation series of 1924 and that of 1925. Furthermore, after twenty-four days the brown blight *Colletotrichum* had produced disease in only two cases out of forty, compared with thirteen successful inoculations out of twenty with the Berry Disease fungus.

The Coffee Berry Disease fungus was also introduced into needle punctures at twenty points on green coffee twigs and into the tissues of thirty-five coffee leaves of varying age. The leaves and twigs were bagged as before after inoculation. Ten and twenty controls respectively were kept in which the treatment was identical except for the omission of the inoculum. The results at the end of twenty-four days were in every case negative and hence in keeping with the observed symptoms of the disease.

It has already been stated that acervuli were frequently found on the dried pulp of berries affected with Coffee Berry Disease and that these fructifications could not be distinguished microscopically from *Colletotrichum coffeanum* Noack. As the result of work with pure cultures on several different media, certain constant differences have been observed between the fungus of Coffee Berry Disease and *Colletotrichum* from other coffee sources. In the course of this investigation, several parallel series of cultures were made of *Colletotrichum* derived from coffee leaves, twigs, berries affected with brown blight and berries affected with Berry Disease. The chief media used were coffee bean extract agar, carrot agar and coffee wood blocks. The cultures were always inoculated within a few minutes of one another and all of one series were grown simultaneously in the same incubator. The agar media cultures in such series were always from the same batch of medium and the coffee wood blocks were made from the main stem of the same coffee bush.

No two of the fungi showed identical types of growth on any of the media but the differences between the *Colletotrichum* from twigs and that from leaves were very small. Incidentally it may be mentioned that *Colletotrichum* from both these sources has at various times given rise, in cultures, to perithecia, the asci and ascospores of which agree closely in form and dimensions with *Glomerella cingulata* (Stonem.) Spauld. & v. Schr. The brown blight fungus from coffee berries did not reach a perithecial stage nor did that of the Coffee Berry Disease *Colletotrichum*, unless certain sterile sclerotia-like masses which occasionally occurred may be considered as attempts at perithecial formation. This last fungus was also characterised by the lack of formation of definite acervuli, but this character was

too inconstant in the other fungi also to be of much value. The form and dimensions of the conidia varied for all four fungi on the different media but the range of variation and the average dimensions were so much alike that they were useless as a means of differentiation.

In one particular, namely the colour of the mycelium, however, the Coffee Berry Disease *Colletotrichum* always differed markedly from the rest. These all appeared perfectly white to the naked eye throughout their growth on all media used, whereas the former fungus invariably became coloured after the first two or three days' growth. This coloration differed with the medium but was always constant with each particular medium. The change from white to colour appeared to coincide with the formation of the first conidiospores. On carrot agar the first change was to green, and, with the growth in diameter of the mycelial colony, there was a gradual enlargement of the green area from the centre outwards, always leaving a narrow periphery of white as long as active growth was taking place. With age the green darkened until eventually the culture was almost black and of a uniform tint.

The colour change on coffee wood blocks was through dark green to practically black with a white margin while active growth lasted. On coffee bean extract agar the changes were not so distinct owing to the dark colour of the medium, but various shades of grey preceded the eventual blackish colour. The Coffee Berry Disease *Colletotrichum* showed a great tendency to form dark brown chlamydospores on the drying out of the media. These were very similar in appearance to the appressoria produced on germination of the spores and were probably largely the cause of the very dark colour of the old cultures.

In connection with the above-recorded colour differences exhibited by different forms of *Colletotrichum* from the same host, it is interesting to note that Burger<sup>(3)</sup> found much the same differentiation amongst different strains of *Colletotrichum gloeosporioides* (Penz.) Sacc. He also found that spore measurements varied with the medium and that the strains were not all affected alike. During the investigation numerous measurements were made for comparison between the conidiospores of the fungus causing the Coffee Berry Disease and those of *Colletotrichum* from a coffee leaf affected with brown blight. Table II, in which the former fungus is represented by the abbreviation "CBD" and the latter by "CL," gives the measurements on three different media. Of these three media, the coffee wood not only bore noticeably longer spores in both fungi but the spores were also more profusely produced than on either of the other media.

Table II. *Measurements in microns of conidiospores of Colletotrichum from two different coffee sources grown on various artificial media.*

Fungus	Medium	Conidiospore measurements
CL	Coffee bean agar	12.1-18.9 $\times$ 4.4-5.9 (exceptional spores up to 22). Average 14.5 $\times$ 5.2
CBD	" "	11.0-18.9 $\times$ 4.6-6.4 (exceptional spores up to 22). Average 15.2 $\times$ 5.5
CL	Coffee wood blocks	12.5-23.0 $\times$ 5.1-6.6 (exceptional spore 27.5). Average 16.5 $\times$ 5.7
CBD	" "	12.8-25.3 $\times$ 5.1-6.8. Average 18.7 $\times$ 5.7
CL	Carrot agar	13.2-20.9 $\times$ 5.1-6.4. Average 15.4 $\times$ 5.7
CBD	" "	12.3-18.7 $\times$ 5.1-6.8. Average 15.6 $\times$ 5.9

Several series of hanging drop cultures were made to compare the germination stages of spores of the two fungi. The media used were two per cent. glucose solution and tap-water respectively. The chief points noted are shown in Table III.

It will be seen that in the glucose solution, where growth conditions were more favourable, slight but quite definite differences occurred. The most noticeable of these were the measurably finer hyphae of the Coffee Berry Disease *Colletotrichum* and the greater tendency of the latter to form chlamydospores, a feature already noted in connection with growth on solid media. The coffee leaf *Colletotrichum*, on the other hand, produced spores more profusely but the difference in this case was not quite so striking.

The dark brown bodies formed on the germ-tubes are here called "appressoria" and those produced on the lateral branches "chlamydospores." This distinction is made for convenience only and is not intended to indicate any real difference in character.

Since the fungus from the diseased berries has so far failed to produce a mature perithecial stage on any of the media on which it has been grown and as perithecia have not been found in the field, it is impossible to be very definite as to its exact relationship to other forms of *Colletotrichum* on coffee. The characters of the acervuli on natural infections on coffee berries, however, are so closely in accord with those of *Colletotrichum coffeanum* Noack that it seems impossible to regard the former as a distinct species. The author prefers therefore to regard it for the present as merely a strain of *Colletotrichum coffeanum* Noack. Sufficient evidence has not been collected to show that the forms of *Colletotrichum* on coffee constitute a polymorphic species such as Burger considers *Colletotrichum gloeosporioides* Penz. to be, but there are indications that such is the case.

Table III. *Comparison between the germination stages of spores of Colletotrichum from a coffee leaf and from a coffee berry affected with Coffee Berry Disease.*

Culture liquid	Age of culture in hours	A. <i>Colletotrichum</i> from coffee leaf	B. Coffee Berry Disease <i>Colletotrichum</i>
Sterile tap water 22° C.	21	Very few spores germinated. No appressoria. Single germ tubes	Several spores germinated, a few with two germ tubes. Occasional germ tubes with an appressorium
	43	Germination general. Appressoria uncommon. Lateral branching sparse. No chlamydospores	Germination general. Appressoria numerous. Lateral branching sparse but some branches terminated by chlamydospores
	66	Hyphae much longer than in B. Very few, scarcely pigmented chlamydospores	Chlamydospores numerous, as many as five on one mycelium. Very few spores formed
	92	Chlamydospores in moderate numbers but most of them almost colourless. Spores fairly numerous	Dark brown chlamydospores terminating practically all lateral branches. Spores few
	120	Most chlamydospores dark brown but not so numerous as in B	As at 92 hours except hyphae adjacent to chlamydospores becoming tinted brown
	138	No change	No change
Sterile 2% glucose solution 22° C.	7-15	Germination begun. Some spores septate. Germ tubes stouter than in B	Germination begun. Some spores septate
	24	Appressoria rare and indistinct	Appressoria formed where in contact with cover glass. Occasional appressorium already germinating. Elsewhere in drop as in A, except hyphae not so stout
	48	Branching vigorous, mycelium visible to naked eye. No spores yet formed. Appressoria as at 24 hours	Branching vigorous on mycelium which formed no appressoria. Visible to naked eye. Mycelium with appressoria little branched. Sparse production of spores
	72	Spores numerous, frequently more than one from same conidiophore. Moderate number of chlamydospores where in contact with cover-slip	Spores few and nearly all from lower part of drop. Chlamydospores numerous in contact with glass
	96	Growth ceasing. Total spore production much greater than in B. Hyphae 1-7 $\mu$ diameter	Growth ceasing. Chlamydospore production much greater than in A. Hyphae 1-5.5 $\mu$ diameter

Spraying with Bordeaux mixture or carbide Bordeaux mixture (an equally efficient substitute in which lime is replaced by calcium carbide when good lime is not available) has proved effective in controlling the Coffee Berry Disease. Spraying, however, is a tedious operation in large plantations, and experiments are in progress to test the value of certain manures in increasing the resistance of the bushes to the disease. It has also been observed that some types of bush show a considerable degree of immunity to the disease and seed selection work is therefore being carried out. Some years must elapse, however, before definite results can be looked for from this work.

#### SUMMARY.

1. A new and destructive disease attacking green coffee berries was reported in Kenya in 1922.
2. The symptoms of the disease and the conditions under which it occurs are described.
3. Certain other conditions found in coffee which might be confused with the present malady are mentioned.
4. A fungus, whose fructifications on the affected berries cannot be distinguished from those of *Colletotrichum coffeanum* Noack, has been isolated from diseased material and has been proved by inoculation to be the causative agent.
5. Comparisons have been made between the growth in artificial culture of this fungus and that of other strains of *Colletotrichum* from coffee. The possible relationship between these is briefly discussed.
6. Methods of controlling the disease are indicated.

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## ON A CORE ROT AND PREMATURE FALL OF APPLES ASSOCIATED WITH *SCLEROTINIA FRUCTIGENA*.

*By W. J. Dowson.*

(With Plate V and 1 Text-fig.)

### I. OCCURRENCE.

THE present paper deals with a curious internal rotting and the premature fall of certain varieties of apples which occurred at the Royal Horticultural Society Gardens at Wisley, Surrey, in the summer of 1925. Towards the middle of July of that year it was noticed that the fruit of the trees of the early-ripening apple "White Transparent" had fallen in large numbers, and that of those remaining on the trees a few were yellower than the rest. At the time it was considered that this variety had ripened a little earlier than usual.

The largest of the fallen apples were picked up with a view to eating, but on biting into them every one was found to have a soft, rotten and brown core. Closer inspection showed that most of them were marked by a small, soft, brown patch, about half an inch in diameter, either surrounding the calyx, or at the base, about the insertion of the stalk. Some had no brown patch or other external blemish at all.

When cut in half longitudinally, every apple, whether bearing a brown patch or not, was found to be rotten at the core.

The yellower, and apparently ripe apples, still attached to the trees were in the same condition. The rot was most extensive in those with the basal marking, and least so in those without any external sign. In every fruit examined the rot involved not only the upper part of the core, but always the tissue between this and the calyx, and in those apples with a basal brown patch, the rot extended from just beneath the skin of the eye, right through the core, down to, and round the insertion of the stalk including the skin. In every specimen cut open the seeds were fully formed but were nearly all soft and rotten, the parchment chamber was discoloured brown and a fluffy white mycelium was sometimes present in the core cavity, either attached to the walls or to the seeds. In the more advanced stages of the rot a good deal of the flesh outside the core and extending to the exterior was in a similar rotten condition. The boundary separating the sound from the diseased tissue was quite sharply marked, not merely by the contrast in colour but also by a difference in consistency (see Plate V, fig. 2).

A thorough search of the orchard for any other varieties which might be similarly affected led to the discovery that the varieties "Domino," "Langley Pippin" and "Duchess of Oldenberg" also exhibited the same premature fall associated with a core rot, but to a much less degree than "White Transparent."

All the four varieties ripen early and their fruits mature during August and September; in addition they all have soft, mealy flesh.

Microscopic examination of the soft discoloured tissue taken from various parts, and from many apples revealed the presence of a copious mycelium consisting of hyphae of very variable thickness ( $3\mu$ – $19\mu$ ). In a few specimens bacteria were also present in addition to the mycelium.

## 2. PRODUCTION OF *MONILIA* TYPE OF CONIDIA ON THE DISEASED APPLES.

On July 15th a number of fallen apples of "White Transparent" and "Domino," some with and some without the brown external patches were gathered from the orchard and all except ten cut open. These ten were placed in a box and kept in the dark, the rest were kept on the laboratory bench in the light.

On August 15th some of the cut-open apples had turned completely brown and had produced pustules of what appeared to be *Sclerotinia* (*Monilia*) *fructigena*. Such apples were removed and placed in a separate box. As time went on more and more of the cut apples turned brown and bore pustules of spores identical in appearance with those of the brown rot fungus.

The ten apples kept in the dark did not form pustules and after a month were much shrivelled in appearance, brown nearly all over except for a few greenish areas of irregular shape, and soft to the touch. A few of them were placed outside in the open, partly buried in sand contained in a flower pot; the remainder were kept in the dark and at the time of writing are completely mummified, grey in colour and without any pustules.

## 3. ISOLATION OF THE MYCELIUM.

Two plates of Brown's agar<sup>(1)</sup> were inoculated with pieces of the soft discoloured tissue taken with sterilised forceps from two freshly collected apples which had been cut open for the purpose with a flamed knife. The plates were inoculated on August 12th, and on the 14th, with the aid of a lens, colourless hyphae could be distinguished growing out from all the pieces of diseased apple. By August 17th fairly large growths measuring one inch in diameter had been produced. One of the plates

was then uncovered and examined under the microscope when the presence of both conidia and microconidia was observed. The latter, in considerable quantity, were arranged in parallel chains arising from a single conidiophore and presented the appearance of a *Penicillium* under the low power of the microscope.

Far less numerous were the conidia, also arranged in single or branched chains. These were of the *Monilia* type and corresponded in size with those of *Sclerotinia* (*Monilia*) *fructigena*.

Careful examination showed that the microconidia were produced, one at a time, from the tip of a short conidiophore, and their appearance agreed with that of the genus *Sclerotinia*. Nowhere, and at no time, on these particular isolation plates did the characteristic pustules of *Sclerotinia* (*Monilia*) *fructigena* develop. From these plates subcultures were obtained on steamed potato plugs in tubes, which did eventually produce yellow pustular growths of the *Monilia* type observed by Wormald<sup>(2)</sup>.

Cultures of undoubted *Sclerotinia* (*Monilia*) *fructigena*, obtained from ripe fruit in the same orchard, when grown on this medium were almost identical in appearance with the above; as were also the cultures from the *Monilia*-like pustules which developed on the cut-open diseased apples. On the three sets of cultures pustular patches, buff in colour, were formed on the upper surface of the potato plugs which were otherwise covered with a fluffy white mycelium.

#### 4. INOCULATION EXPERIMENTS.

The presence of the soft brown discoloration either at the stalk or at the eye end of the fruit, together with the thick septate hyphae in the rotted tissue, which when isolated produced the conidial forms of the brown rot fungus, suggested that this indeed might be the cause of the disease, either alone, or in company with other organisms.

Accordingly, it was decided to find out what the effect would be when sound apples were inoculated with the three strains of *Sclerotinia* (*Monilia*) *fructigena* already mentioned.

A large number of inoculations were made during August using apples of many different varieties, some quite small and immature, but the majority were normal apples taken from trees which ripened their fruit somewhat later than the four affected varieties. The results were so uniform that it is not necessary to give them all in detail.

The inocula used were (1) conidia of *Sclerotinia* (*Monilia*) *fructigena*, (2) the similar conidia produced on cut-open diseased apples mentioned above, (3) small pieces of the rotted tissue,



(4) mycelium taken from pure cultures of *Sclerotinia* (*Monilia*) *fructigena*, and (5) mycelium taken from pure cultures of the isolated fungus. The apples were inoculated either (a) beneath a small triangular flap of skin, or (b) on to the core by means of a hole drilled right through the apple with a cork borer, or (c) beneath a conical piece cut from the eye and afterwards replaced, or (d) by means of a needle-stab down through the eye and varying in depth from an eighth to half an inch.

With the exceptions of (b) and (d), the result of inoculation was uniformly the same however performed. The first sign of infection occurred from two to three days after inoculation when a brown discoloration appeared at the place where the inoculum had been introduced. On the following day one or more pustules of the *Monilia* type made their appearance and in the course of a few days the whole apple became soft, brown and covered with pustules.

With method (b) the process took a little longer, three to four days for the appearance of browning and four to five for the production of the first pustule. In (d) the method of introducing the inoculum (conidia in this instance) was regarded as approaching as nearly as possible that which might actually have happened in nature: hence the inoculations under this heading may be given in detail.

*Inoculation No. 10 made on August 27th, 1925.*

Six apples were chosen and pierced through the bottom of the calyx depression as near to the centre as possible by plunging a hot needle down to a distance of about half an inch. A drop of sterilised water containing a suspension of conidia derived from an apple previously inoculated with *Sclerotinia* (*Monilia*) *fructigena*, was placed, by means of a pipette, in the calyx cavity of five of the apples; the sixth received water only and served as a control. The apples were placed on the laboratory bench and covered with a bell-jar.

On August 31st a drop of distilled water was added to the eyes of the six apples but no sign of infection was then evident.

On September 2nd, *i.e.* six days after inoculation, three of the apples bore an irregularly shaped brown patch surrounding the calyx, and all five inoculated apples were found to be soft about this region when pressed with the finger. On the same date all the five were cut open and carefully examined. The tissues extending from the calyx to the base of the core in a vertical direction and past the core in a horizontal direction were soft and discoloured brown. The vascular bundles were more deeply stained brown and were noticeable beyond the discoloured flesh, suggesting that the hyphae had travelled

along the bundles in advance of the mycelium in the rest of the tissue. One marked difference between the rot so produced and that of the naturally infected apples was the diffused edge of the rot in the former as contrasted with the much more sharply delimited margin in the latter. Otherwise, in this experiment the rot produced was closely similar to that observed in nature. The sixth (control) apple remained sound.

*Inoculation No. II made on September 3rd, 1925.*

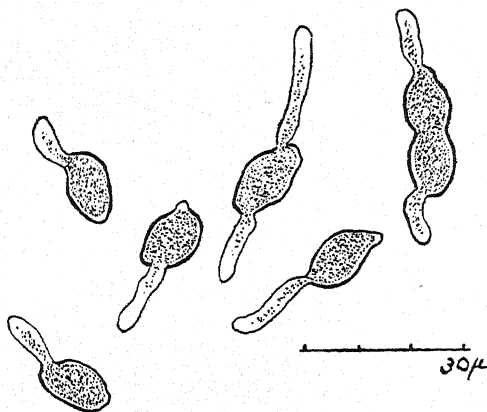
This was a repetition of No. 10 with the exception that instead of making a stab half an inch long, the skin at the bottom of the eye was just pierced and no more. Subsequent examination showed that the greatest depth to which the hot needle had sunk was an eighth of an inch. The inoculum in this experiment was a suspension of conidia taken from a pustule which had formed on one of the cut-open diseased apples kept in the laboratory.

On September 15th one of the five apples bore a large brown patch round the calyx and had begun to produce pustules: none of the others showed signs of infection and when cut open no trace of such could be found.

In a similar experiment in which no wound at all was made, the inoculation drops being placed in the unwounded eyes, no infection resulted.

#### 5. GERMINATION OF THE CONIDIA.

Two observations of interest were made with reference to the germination of the conidia. By means of a series of hanging-drops of various media, the germination of the conidia of the



Text-fig. 1. Conidia of *Sclerotinia fructigena* two and a half hours after sowing in a hanging-drop of Brown's agar.

fungus isolated from the rotted apple tissues, was compared with that of *Sclerotinia* (*Monilia*) *fructigena*. No difference between the two could be detected. On drops of Brown's agar and in tap water containing apple juice, the conidia of both strains began to germinate in the surprisingly short time of sixty to ninety minutes, but no germination was observed to occur in distilled water or tap water alone. The presence of organic substances seems to be necessary for the production of germ tubes although this point has not yet been investigated in detail. Germ tubes were produced from any part of the conidium (see Text-fig. 1).

#### 6. RESULTS OF THE FOREGOING OBSERVATIONS AND DISCUSSION.

That the mycelium contained in the soft and discoloured tissues of diseased apples belongs to *Sclerotinia* (*Monilia*) *fructigena* follows fairly clearly from the observations and inoculation experiments described above. As to whether this fungus was the sole cause of the rot, or, whether other organisms, e.g. bacteria, were also involved, the evidence so far to hand is not conclusive. In none of the inoculations was a rot produced exactly like that observed in naturally infected apples in which the advancing edge of the rot was more sharply marked than in the artificial inoculations.

This may have been due to the means of entry of the fungus in the first instance, and also to the condition (immaturity) of the infected tissues. So far, there is no definite evidence as to when and how infection occurred; though the fact that in every specimen examined the upper portion was infected right up to and often including the skin of the eye, indicates the probability that the fungus had gained entrance at this end.

The apples may have been infected at an early stage of development, possibly even during the flowering period when the blossoms may have been visited by insects bearing spores of the fungus. This however implies the presence of conidia at an unusual season of the year, and yet it does not seem likely that the fungus had been introduced in any other way than by spores.

It is just possible that infection may have arisen from ascospores from old mummies. The apothecial form of *Sclerotinia fructigena* has been known on the continent since 1905(3) but has not yet been recorded for this country.

Experiments have been planned, with the object of obtaining more information as to how, when and where *Sclerotinia fructigena* gains entrance to apples, so as to produce an internal rot.

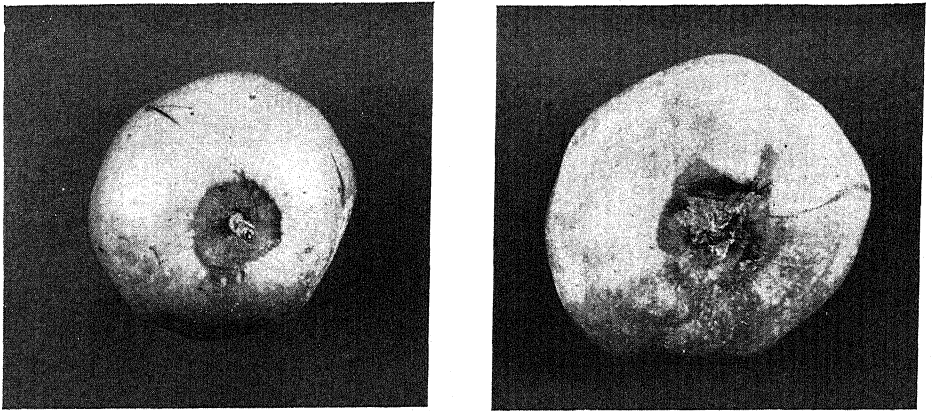


Fig. 1.



Fig. 2.



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## EXPLANATION OF PLATE V.

Fig. 1. Core rot of apple "White Transparent." On left, view of stalk end showing soft brown patch. On right, view of eye end showing soft brown patch.

Fig. 2. Top. Views of the same two apples as seen when cut in half. Bottom. An apple without any mark upon the outside as seen when cut in half. Note the rotten tissue between the base of the calyx depression and the core in all three. The only mark on No. 1 (top left) was that shown in Fig. 1 (left), the *skin* of the apple appeared quite sound, but the tissue just beneath was rotten.

## SUCCESSIONAL DISEASE IN PLANTS AS SHOWN IN WILLOW RODS.

By N. L. Alcock, F.L.S.

(With 1 Text-fig.)

THE course of disease in plants, in the light of recent investigations bears a striking resemblance to that of the micro-organisms whose attacks upon the human body are rarely successful when the latter possesses normal powers of resistance. We are apt to forget how close is the resemblance between the two forms of life, animal and vegetable.

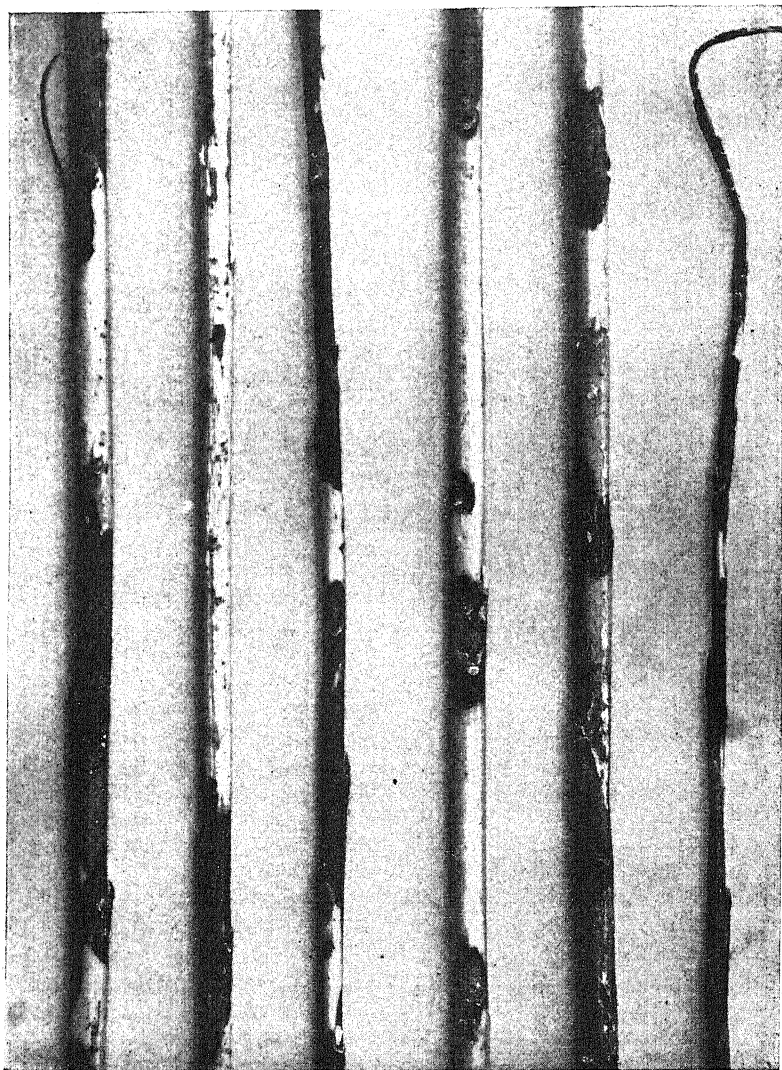
Resistance to disease in both cases fails for three main reasons: (a) an unsuitable environment, mismanagement or injury, (b) neglected attacks of minor ailments, (c) and only occasionally by the attack of a specific disease of remarkable virulence. This finds a good illustration in the four diseases here described.

(1) The Willow Scab, actively parasitic like apple scab, attacking willow rods and much assisted by faulty methods of cutting.

(2) The three diseases in succession on the crack willow, which show how one disease leads to another and how the weakened plant ceases to have its full power of resistance.

The Willow Scab, *Fusicladium saliciperdu* Tubeuf, is a strong parasite, the most actively parasitic perhaps of the four.

The second, *Cryptomyces maximus* (Fries) Rehm, is also a parasite, *Scleroderris fuliginosa* (Pers.) Karst. a semi-parasite, *Myxosporium scutellatum* (Otth.) Petrak hardly a parasite, mostly



*Fusicladium saliciperdatum* Tub. on willow rods.

a saprophyte, but all were injuring the trees on which they were found.

WILLOW SCAB (*Fusicladium saliciperdu*m (All. & Tub.) Tubeuf (8, 9, 10). This disease was noted on willow rods by a market gardener in Lanark and the specimens sent to the Botanic Garden, Edinburgh. They were suffering from killed and curled tips, striking black blotches on the stem, giving a piebald appearance, and in some cases an extensive die-back.

Although osiers are little grown in Scotland, the growers and nurserymen frequently grow a few willows and cut them over for their own use. There is no reason that I know why willows, which grow particularly well in Scotland, should not be grown commercially.

These rods were apparently *Salix alba* var. *vitellina*. A parasitic fungus was present and after examination it was decided that the disease was that known on the Continent as Bark Scorch of Willows, a troublesome disease of willow rods grown for culture.

*Fusicladium saliciperdu*m (All. & Tub.) Tub. is very closely related to the fungus causing apple scab, *Fusicladium dendriticum* Fckl. (*Venturia inaequalis* Aderh.). The perfect stage of *Fusicladium saliciperdu*m is said to be *Venturia chlorospora* (Ces.) Karst. This form, as far as I know, has not been seen in Great Britain. The disease attacks many species of willows, including *Salix alba*, *S. aurita*, *S. caprea*, *S. cinerea*, *S. cuspidata*, *S. fragilis*, *S. mollissima*, according to Rehm (3, 776); also *S. nigricans* and *S. pentandra* according to Tubeuf (11). It has been noted in Bavaria and other parts of Germany, in Denmark, and recently in the Netherlands (12), where it has caused a disease, particularly of the weeping willow. The ordinary weeping willow in the Netherlands is *Salix vitellina* var. *pendula*—not *Salix babylonica*, as in the British Isles.

The disease appears first as dark blotches on the leaves, covered with a velvety pile, the dark olive-coloured area sharply contrasting with the green of the healthy leaf. These spots resemble the spots on the apple leaf caused by apple scab, and are similarly covered with the fungus producing its conidial fruiting stage. The upstanding conidiophores, bearing at the apex the conidial spore, give the velvety appearance to the diseased area. The spore is large, varying in size from  $15 \times 5\mu$  to  $22 \times 10\mu$ , but usually about  $18 \times 6\mu$ . When first formed this spore frequently appears non-septate, but when mature is typically 1-septate with a constriction at the septum, hence the statement frequently made that the spore is shaped like the sole of a shoe. The spores are exceedingly variable in size and shape. These early spots on the leaf give rise to further infection in several ways:



- (1) The spores are carried to and infect another leaf.
- (2) The spores are carried or blown to the tender tips of the young shoots, and, infecting the soft tissue, cause a die-back; the shoot turning black, the tip sometimes curling in a hook as in *Monilia* tip-wilt of plum.

- (3) The parasite spreads over the leaf and travels along the mid-rib and down the petiole to the leaf-base and infects the cortex of the stem at the junction of the leaf, spreading above and below. The withered mid-rib may be seen on several diseased twigs still attached by the withered petiole to the stem.

The infection by the fungus causes the bark to turn black, and the disease thus forms striking long-shaped patches on the bark. The black discoloration is sharply marked out on the yellow bark of the rod, and an infected twig looks strongly piebald. In a bad case the infected areas run together until the whole rod is black.

On these black areas small pustules are formed under and pushing up the epidermis. They are often roughly set in an oval, a half circle being found some way above the point of infection, and a half circle some way below—others are scattered. They are of varying size but many are from one-fourth to one-third of a millimetre. These pustules are formed of a compact mass of fungal tissue which can go through a dormant period in a similar manner to the behaviour of the resting-stage of the cushion of apple scab. It is probably in these cushions that the fungus over-winters. When the conditions are favourable the mass of hyphae grows and forces up and finally ruptures the bark. At first the pustule has a shiny black appearance, but when the cushion has burst through the bark, it becomes covered with spores and short conidiophores, and appears dusty and dark olive-brown.

These spores act again as a source of infection, and judging by the similarity of the form on the willow twig to the dormant stage of apple scab on such apples as Cox's "Orange Pippin" this is the origin of the early infection on the leaves of the willow.

In dealing with the other bark troubles of willow, such as *Physalospora gregaria* Sacc., often called willow canker, which is common in Somerset on basket willows (*S. triandra*, *S. viminalis*, etc.), it has been found that the disease is very much increased by the habit of cutting the rod of the required length only. In other districts where the custom has been to cut right out down to the stool and trim the rod afterwards, the disease has been much less troublesome. In dealing with osier willows, or stools of willows kept for commercial or useful purposes in nurseries, the rod should be cut as low down and as near to

the stool as possible. Any long stub left forms a home for the pustule form of this scab. Long stubs should therefore be avoided.

All diseased twigs should be cut out so far as possible during the winter and burnt before the dormant pustules awaken and form spores. If the value of the willow or the usefulness of the withies will warrant the expenditure of money and labour, a winter wash of copper sulphate—four pounds of copper sulphate to one hundred gallons of water—could be given in the winter, or lime sulphur—one gallon to twenty-nine gallons of water—might be tried in the spring. In the case of osiers, spraying the stumps with Bordeaux mixture after cutting might be tried.

The three other diseases were acting in succession on the crack willow (*Salix fragilis*).

*Cryptomyces maximus* (Fr.) Rehm (1,246; 2,707; 3,107; 4; 5), the first of these, is a distinct parasite. This fungus forms long black cushions on the green or yellow-green bark of the willow twig. The cushion may be six or eight inches long, raised above the bark, with a curious blistered appearance at the edge of the cushion, the black colour of which contrasts strongly with the pale tint of the willow shoot. The top of the long cushion is flat and silvery black. At a little distance the infected branches look as if they had been scorched with fire. The fungus is parasitic on living twigs of various willows, which it sometimes entirely girdles. It seems that the apothecial cushions burst through the young green bark in the late summer or autumn, maturing in spring. The hymenium takes up about a third of the whole thickness of the cushion. It is of an olivaceous black on the outside and of a dark colour within. The lower part of the substance is white. The asci are very long with eight spores. The spores are oval, obtuse, one-celled, with a central oil drop, colourless or slightly yellowish,  $20-26\mu \times 10-18\mu$ . Paraphyses are present and abundant, appearing like brownish threads among the asci and very long. Rain causes the black cushions to swell and scale off, leaving long scars in the bark. The disease generally spreads over branches, even to the small twigs, frequently causing the death of the twig above the infection.

*Scleroderris fuliginosa* (Pers.) Karst. (1,250; 2,595; 3,210; 6), the second disease, is very destructive in action when it has found a means of entry in a weakened host. In this case it had entered *via* the scars and lesions made by the *Cryptomyces*. Other diseases, such as the Yellow Rusts, *Melampsora* spp. also form a means of entry for further fungus trouble on the willow.

Dr Malcolm Wilson, for whose help in this work I am much indebted, gives an interesting case where a series of fires had been made in the forest in the disposal of slash. These fires had been too near to some Douglas Firs, which are, in any case,

particularly susceptible to scorching. Up each tree was a narrow strip of scorched bark, and each scorched area was covered with the fructifications of *Scleroderris livida*, a fungus very like the one on the willow. The circle round where the fire had been was distinctly marked by the trees bearing the fungus.

*Scleroderris fuliginosa* occurred round the edges of the flat cushion, formed by *Cryptomyces maximus*, as a collection of large brown or black pycnidia. On other branches, weakened by the *Cryptomyces*, this fungus has appeared above the first disease.

The pycnidia are very large, up to a quarter of a millimetre, often with a flat top, in some cases almost square, opening by a pore at the top. They grow closely huddled together. They have rather the appearance of turnip or rape seed set round and encroaching over the black flat cushions of the *Cryptomyces*. The spores contained within the pycnidia are hyaline, 20–30  $\mu$  long, with three or four divisions. They are borne on short conidiophores, set all round the inside of the pycnidium. These conidiophores remain with the appearance of a fringe when the spores are washed out. Following these pycnidial fructifications an apothecial stage occurs, forming in and occasionally on the top of the pycnidial stage.

These apothecia are at first squatly round, and closed at the top with a dent in the summit, often with a stout stem-like base. The disc is greyish white, about a quarter to three-quarter mm. across, but varying in size, the exterior brownish black. The asci are very long and club-shaped, with the needle-shaped spores arranged in a parallel bundle within the sac. The ascospores are very long, up to 60  $\mu$ , hyaline, pointed at both ends, thin, mostly straight, with seven or eight divisions. Slender colourless paraphyses are present. This fungus is provisionally identified as *Scleroderris fuliginosa* (Pers.) Karst.

*Myxosporium scutellatum* (Otth.) Petrak (7). After the young shoots of *Salix fragilis* had been considerably weakened by *Cryptomyces maximus* and *Scleroderris fuliginosa*, *Myxosporium scutellatum* appeared on the dead and injured twigs practically as a saprophyte but increasing the areas of injury. These dead areas may be a foot or more in length in which case the tree may be greatly disfigured and useless for many purposes. The fungus is perennial, living from year to year in the bark and causing a fresh diseased area each year. Scattered over the dead bark are innumerable disc-shaped areas about the size of a pin's head. These represent the fructifications of the fungus, and are at first under the epidermis. Later the epidermis ruptures, and the fructifications appear as cup-shaped or saucer-like structures. The bark in many cases tends to peel off readily, carrying the fructifications with it.

In transverse sections the fructifications consisted of a single chamber, though in a few cases the columnar structure was visible in the centre, indicating a division into two compartments.

Dr Petrak, who kindly assisted in the identification, has now placed his fungus in a new genus—*Cryptosporiopsis*.

I am indebted to the kindness of Mr J. Fraser, of the Forest School, Beaulieu, for these specimens of *Salix fragilis*.

It is hoped to carry out inoculation experiments this year and the willows are being grown in the Royal Botanic Garden.

The succession of diseases on the crack willow, *Salix fragilis*, dealt with here, illustrates clearly how one disease can lead to another, and how a plant in a weakened condition ceases to have the full power of resistance.

Diseases may be caused by such virulent parasites that the healthiest of plants will succumb. But a good many fungus diseases depend on lack of vigour in the host. A plant lessened in strength by a strong parasite will frequently become the victim of many half-parasitic fungi. A plant grown in bad conditions, overcrowded, or on an unsuitable site, will suffer from fungal trouble where a tree on suitable soil, at a right elevation, will probably never have a disease unless the virulent specific parasite appears. For example, the White Pine Blister Rust, or the Chestnut Bark Canker, that has destroyed the chestnut forests of the Eastern United States, will attack the most flourishing tree in the best condition and site.

Again, plants are more susceptible to disease at certain times. Seedlings are at a critical stage in their career and will readily succumb to fungus attack under unfavourable conditions. Such operations as transplanting or grafting form times of danger to the plant, when for the moment its resistance is low and enemies have a chance. But one of the commonest dangers is that one fungus, possibly not very serious in itself, may form a means of entry for another which will do great harm.

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## REVIEWS.

*The Romance of the Fungus World.* By R. T. and F. W. ROLFE. Chapman and Hall, Ltd. xiii + 309 pp. 12s. 6d. net.

Under the above alluring title two of our members, Messrs R. T. and F. W. Rolfe, have gathered together in one volume miscellaneous information of a kind that should attract readers outside the charmed but very restricted circle of mycologists.

These, too, will find the book worth having, especially if they are in search of the padding that is indispensable in a popular lecture. After all, one cannot expect an audience to be "thrilled" over the subtle differences of species, but the dreadful lethal habits of *Amanita* this, and the less deadly but intoxicating ways of *Amanita* that, have a perennial interest. Such information is supplied abundantly in this book. There are chapters on fungi in Folk-lore, Literature, Medicine, Industry, etc. The photographs are very good; they are mostly by Mr A. E. Peck, and he must be delighted to see them so well reproduced. Mr Ramsbottom bestows his blessing in a short Foreword.

A. A. P.

*A Text-Book of Organic Chemistry, Historical, Structural and Economic.* By JOHN READ. G. Bell and Sons. xii + 680 pp. 12s. 6d. net.

Mycologists and botanists in general have frequent occasion to refer to books on Organic Chemistry. The new book on this subject by Professor J. Read is probably the most suitable for workers in biology that has yet been published, for the author has devoted special attention to the substances such as alcohols, sugars, starches, celluloses, essential oils, etc., which play so important a part in plant metabolism and which are of such great importance economically. Frequent reference is made in the book to the bio-chemical activities of micro-organisms. Apart from its intrinsic merit to students of biology the book is written in an extraordinarily interesting manner and contains a fascinating account from the historical standpoint of the rise and development of the study of Organic Chemistry.

F. T. B.

## PROCEEDINGS.

AUTUMN FORAY AND ANNUAL MEETING, DUBLIN.

21st—26th September.

AUTUMN FORAY FOR LONDON STUDENTS, OXSHOTT. 10th October.

FORAY WITH ESSEX FIELD CLUB, EPPING FOREST. 17th October.

FORAY WITH BRITISH ECOLOGICAL SOCIETY, BURNHAM  
BEECHES. 24th October.

MEETING. UNIVERSITY COLLEGE, LONDON. 21st November.

S. DICKINSON. A new method of isolating single spores.

W. J. DOWSON. Fall of apples associated with a core-rot due to *Sclerotinia fructigena*.

J. RAMSBOTTOM. Fragmenta mycologica. IV.

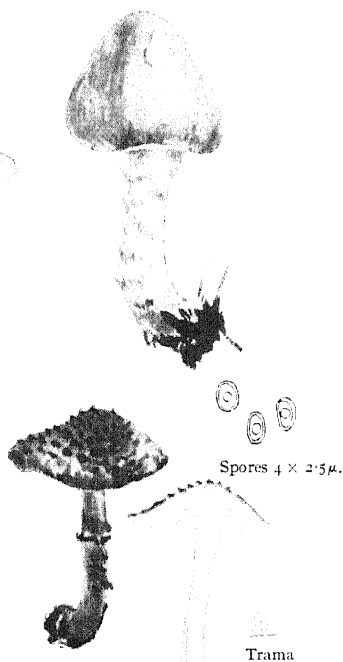
ALEX. SMITH. *Penicillium* diseases of *Gladiolus* and *Narcissus*.

A. L. SMITH. Lichen Dyes.

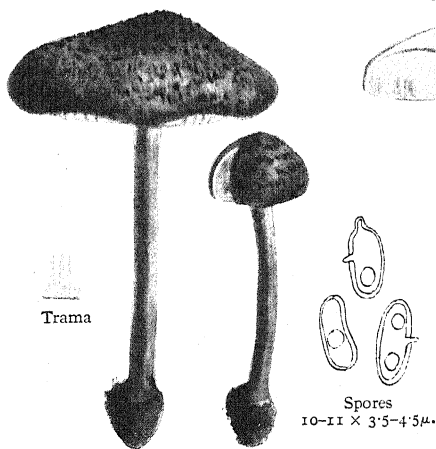
W. BUDDIN and E. M. WAKEFIELD. Life history of a fungus parasitic on *Antirrhinum majus*.



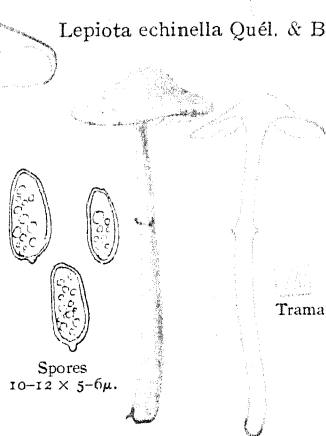
*Lepiota pratensis* (Fr.) Rea.



*Lepiota echinella* Quél. & Bern.



*Lepiota castanea* Quél.



*Lepiota gracilis* (Quél.) Rea.

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(21 Dec 35)

# ON THE LIFE-HISTORY OF A FUNGUS PARASITIC ON ANTIRRHINUM MAJUS, WITH SOME REMARKS ON THE GENUS HETEROSPHAERIA.

(With 8 Text-figs.)

By *W. Buddin and E. M. Wakefield.*

## OCCURRENCE AND DESCRIPTION OF DISEASE.

IN 1917 and 1918 *Antirrhinum*s were received from two different sources showing a leaf disease which appeared to be distinct from anything previously recorded on this host. What was obviously the same disease was noted in 1920 by Miss Cayley and described by her in some detail, though the fungus was not fully identified (1).

Since then the disease has frequently been recorded, and appears to be at present one of the most common and at the same time perhaps the most destructive disease of cultivated *Antirrhinum*s in this country. It does not appear, however, to have been noted outside Great Britain.

A brief recapitulation of the chief characteristics of the disease may be given here.

At suitable temperatures, in about ten days after spraying young and still tender leaves with a spore suspension, there appear the first visible signs of infection in the form of pale green rounded spots, about 5 mm. in diameter. As has been mentioned in a previous note (2), the growth of the mycelium and production of spores of the fungus is active only under somewhat cool conditions, but when once started in a bed the disease spreads very rapidly during spells of cool, moist weather. The leaf spots become more clearly defined owing to the collapse of the affected cells. The subsequent appearance depends to some extent on the variety of *Antirrhinum*, and also upon weather conditions. The central portion of each spot gradually becomes pale and dries out. Sometimes a definite purplish margin may be developed but in other cases there is no such distinction of colour. Sometimes also the dried areas fall out, leaving holes in the leaf, and the appearance is then like that known as "shot-hole" in plums, etc., or as if the leaves had been eaten by an insect (Fig. 1). Although the leaf phase is always the most conspicuous form of attack, in cases of heavy infestation the green stems also develop lesions which eventually become swollen canker-like areas owing to the production of wound cork beneath the pustules of conidia. When the stems



are infected the flower-stalks may be poor and distorted, and the whole plant has a sickly appearance. Eventually, badly attacked plants are killed outright.

Under suitable moist conditions conidia are produced abundantly on both sides of the leaf, and the numerous acervuli are seen easily with the aid of a pocket lens. Under dry, hot conditions, however, the formation of conidia is temporarily restricted. The acervuli are crowded closely on each spot, are of a very pale pinkish tinge and somewhat waxy or moist in

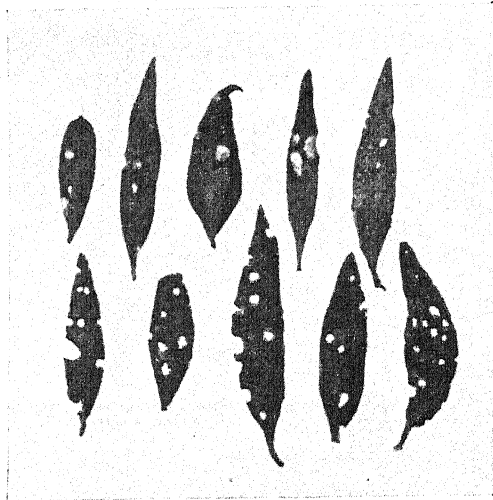


Fig. 1. Leaves of *Antirrhinum majus* showing spots caused by *Cercospora Antirrhini*.

Upper row—early stages.

Lower row—showing subsequent "shot-hole" effect.

appearance rather than pulverulent. Vertical sections show that each acervulus arises just beneath a stoma as a minute cushion of closely woven hyaline hyphae. This cushion bears on its outer surface numerous erect, branched conidiophores, which burst through the epidermis and produce at the exterior the curious conidia (Fig. 2, *a-c*). These are at first narrowly obclavate, curved, for a long time non-septate but finally one- to three-septate (Fig. 2, *c*). At length, sometimes only after separation from the conidiophore, the apical portion becomes drawn out into a long fine awl-like point, while after the shedding of the conidia a second but shorter appendage may appear at the base of the spore (Fig. 2, *c*).

Presumably the conidia are washed away in drops of rain or

dew or in the splashes made by watering, and in this way infection is spread. In no case has any other form of fructification been observed on the living plants, and an examination of dead plants left standing in the beds revealed no fungus which could conceivably be regarded as being connected with the Hyphomycete under consideration.

At the time the disease was first received a careful search was made in the literature, but no mention could be found of any fungus occurring on *Antirrhinum* or related genera which was at all suggestive of this species. The fungus was accordingly described as a new species of *Cercospora*, *C. Antirrhini* Wakef. (3).

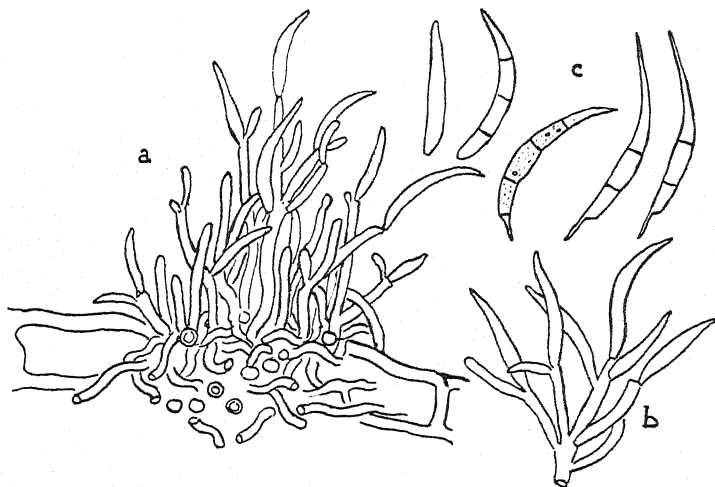


Fig. 2. *Cercospora Antirrhini*. a, Vertical section of acervulus. b, Branched conidiophore. c, Conidia at various stages. ( $\times 850$ .)

The reference to *Cercospora*, as in many cases of Hyphomycetes whose other stages are unknown, was purely a matter of convenience, *Cercospora* being the nearest "form genus" into which the species would fit. It was recognised at the time, however, that the fungus differed in several respects from a typical *Cercospora*, and might later have to be removed from the genus.

#### TEMPERATURE RELATIONS OF PARASITE.

Towards the end of 1923 cultures were started from conidia, and were put on several different media which happened to be available at the time—chiefly Dox's agar, prune agar, and malt extract agar.

At the outset some difficulty was experienced in obtaining abundant growth. Microscopic observations of platings of the conidia on agars revealed the fact that a remarkably high proportion of the conidia failed to germinate, and some rough experiments on the effect of heat on the conidia were therefore carried out.

The conidia germinate slowly and do not lend themselves well to the adoption of the identical technique used by Henderson Smith<sup>(4)</sup> in his careful study of the effect of heat on the spores of *Botrytis cinerea*. The following method was adopted. Tubes of agar were melted and cooled to the desired temperature in a water bath. After the tubes in each batch had attained the temperature of the bath, equal drops of a heavy suspension of conidia of the fungus were added to each tube, the dilution of conidia in the agar maintained, with shaking, at the particular temperature for one or five minutes, and at the end of the period the contents of the tube were poured quickly into a sterile Petri dish. The dishes were then incubated at 18° C., and after the lapse of an ample period (three weeks) to allow of all viable conidia producing colonies the numbers on the plates were counted.

With malt extract agar as the medium in which the conidia were heated the results shown in Table I were obtained. Very

Table I.

Number of colonies of *Cercospora Antirrhini* developing on plates of malt extract agar when approximately equal numbers of conidia are heated at the temperatures given, for the times stated, suspended in that medium.

Temperature maintained in water bath	Time of heating	
	1 minute	5 minutes
41° C.	Approx. 10,000	24
43° C.	Approx. 2,500	1
45° C.	5	Nil
47° C.	Nil	Nil

Table II.

Number of colonies of *Cercospora Antirrhini* developing on plates of prune agar when approximately equal numbers of conidia are heated at the temperatures given, for the times stated, in that medium.

Temperature maintained in water bath	Time of heating		
	Nil (Suspension poured at once)	1 minute	5 minutes
41° C.	Approx. 10,000	—	—
43°, 45° and 47° C.	—	290 Nil	Nil Nil

similar results were obtained in a duplicate set with malt extract agar, and also with potato gelatine, using different suspensions of conidia. Comparison with the curves of Henderson Smith

indicates that the conidia of *Cercospora Antirrhini* in malt extract agar and potato gelatine are killed at an appreciably lower temperature (circa 5° C.) on the average than those of *Botrytis cinerea* heated in distilled water. The effect of the medium is, however, strikingly shown by the results in Table II, obtained by heating suspensions of the conidia in prune agar (Difco). The conidia were from the same heavy suspension as those used with malt extract agar for the results shown in Table I, and drops of equal size were used for each tube. A duplicate set with prune agar, using a different suspension of conidia, gave very similar results. The figures show that when heated for one minute in the medium the average conidium of *Cercospora Antirrhini* will withstand a temperature 2-3° C. higher in malt extract agar or potato gelatine than in prune agar. The results also emphasise the advantage of using a gelatine medium when plating out the conidia of an unknown fungus.

The growth of the fungus in pure culture is extremely slow, colonies started from spores not attaining a diameter of 3 cm. until after the lapse of a month at the optimum temperature. This appears to be at approximately 18° C., but growth is almost as good at a low laboratory temperature averaging about 13° C. No growth at all, either from spore inoculations or of colonies of the fungus, takes place at a temperature of 25° C. or higher, although exposure to 25° C. on agar plates for a month does not kill the organism.

It may be noted here that similar temperature relations were found by Vestergren (5), p. 17) when working with the fungus *Heteropatella cercosperma* (Rostr.) Lind (= *Rhabdospora cercosperma* (Rostr.) Sacc.). In that case temperatures of 25° to 30° C. inhibited conidial formation. The maximum lay between 30° and 35° C. and the optimum at about 20° C. (between 18° and 25° C.). The point will be referred to again in the section on taxonomy.

#### GROWTH ON VARIOUS MEDIA.

Cultures which had been kept for some time were noted during the winter to have produced different growth according to the medium used. On Dox's agar or potato agar the mycelial growth is white and scanty, and closely adpressed to the surface of the medium in a radiating form. Minute pinkish pustules of conidia are developed, on potato agar all over the colony, but on Dox's agar tending to be aggregated towards the centre. No further development was noted on either of these media. On prune agar, however, mycelial growth is rather more vigorous, and in addition to the radially spreading, closely adpressed hyphae tufts of erect filaments consisting of bundles of hyphae

often occur towards the centre of each colony. Conidia are formed abundantly in four days, and at the end of a fortnight there are seen here and there, especially towards the middle of the colony, small black bodies resembling sclerotia.

On malt extract agar the mycelial growth is more compact than on the other media mentioned and conidial pustules develop

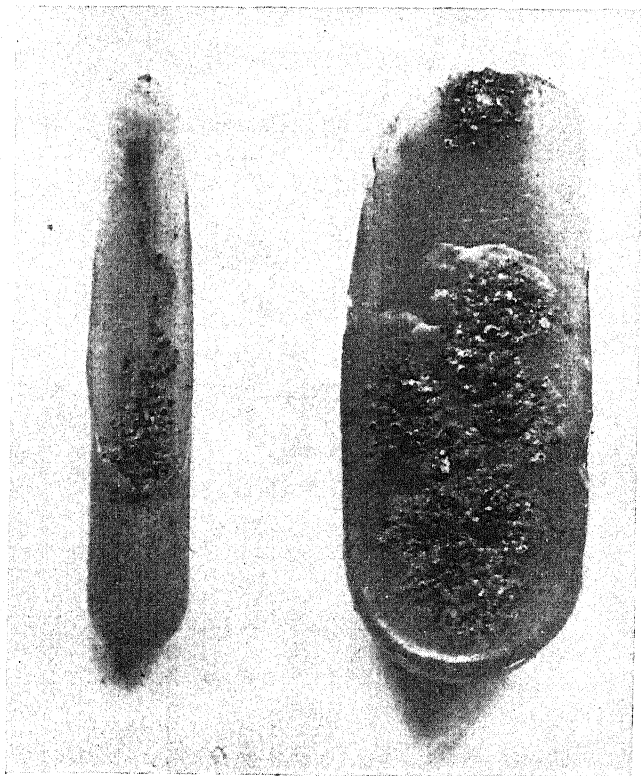


Fig. 3. Two cultures of *Cercospora Antirrhini* on malt extract agar, showing abundant *Heteropatella pycnidia* (nat. size).

abundantly in the middle of the colony. On this substratum also the sclerotium-like bodies occur, but they are more slowly formed than on prune agar (Fig. 3).

Similar bodies were found later to be produced also on sterilised antirrhinum stems.

As these blackish bodies seemed most likely to throw light on the affinities of the fungus, observations were concentrated

on them. At first they were rounded and compressed in form (more or less "bun"-shaped) and quite solid, black externally but composed within of thick-walled, closely interwoven, colourless hyphae. Later it was noted that some of them had opened out above and become discoid, with a slightly raised, torn margin. The appearance suggested that the bodies represented the perfect form of the fungus and that this was to be sought amongst the Discomycetes. Repeated examination of even the most well-developed discs, however, failed to reveal any trace of asci. Cultures showing them were kept until almost drying up, and were exposed to low temperatures outdoors for 24-48 hours. Further, various media and concentrations of media were tried. If left in the tube in which they were formed, the discoid bodies always remained sterile, the erect filamentous hyphae composing the disc eventually becoming much swollen in parts and obviously abnormal. When transferred to another tube, however, and thus supplied with fresh food material and moisture, the supposed hymenial surface immediately produced an abundant crop of conidia, similar to the original *Cercospora* conidia.

#### OVER-WINTERING OF DISEASED ANTIRRHINUMS.

At this point further observation of pure cultures was abandoned, and during the winter of 1924-5 attempts were made to find similar bodies produced in nature. Numerous antirrhinums of several different varieties had been inoculated during the summer and had become badly infected with the disease. Some of these, both at Reading and at Kew, were left standing in the beds; affected leaves and stems of others were placed in empty flower-pots covered with muslin and stood out of doors throughout the winter.

As already mentioned, plants left in the beds gave no result, probably because the stems were not under sufficiently uniform moist conditions. Stems and leaves over-wintered in pots also showed nothing. It was difficult to keep these sufficiently moist and at the same time protected from being eaten by slugs, woodlice, etc., and in London possibly the heavy black fogs of that winter may have had a deleterious effect. At Reading, however, better fortune was met with. In March 1925 numerous decorticated stalks from a large heap of dead Antirrhinums, which during the summer had shown the disease, were found to show an abundance of black Discomycete-like bodies similar in appearance to those formed in pure culture (Fig. 4).

These fructifications proved to be pycnidial only, and although the stems were kept under observation for some months

no ascigerous stage was ever found. From the evidence now obtained, however, it seems certain that the perfect stage, if produced at all, would be a Discomycete. The pycnidial form as found on the over-wintered stems is an undoubted *Heteropatella*, a genus of Excipulaceae, species of which are known



Fig. 4. *Heteropatella* pycnidia developed on over-wintered dead stems of *Antirrhinum* (nat. size).

in other cases to belong to the Discomycete genus *Heterosphaeria*. Apart from the similarity to the bodies seen in cultures, proof of the connection of the *Heteropatella* on the dead stems with the parasitic *Cercospora* was obtained by inoculation of living *Antirrhinum*s with spore-suspensions from pure cultures of the former, when the characteristic disease spots on the leaves were produced.

## MORPHOLOGY.

(a) *Cercospora* stage. It has been already stated that the acervuli of the parasitic Hyphomycetous stage develop beneath the epidermis of the leaf or green stem as small hyaline hyphal masses. From these arise erect branched conidiophores which burst through the epidermis in small tufts and produce the conidia externally. The conidiophores are hyaline, branched from near the base, and  $2-4\ \mu$  in diameter. The conidia arise at the tips of the branches, and are at first non-septate, slightly

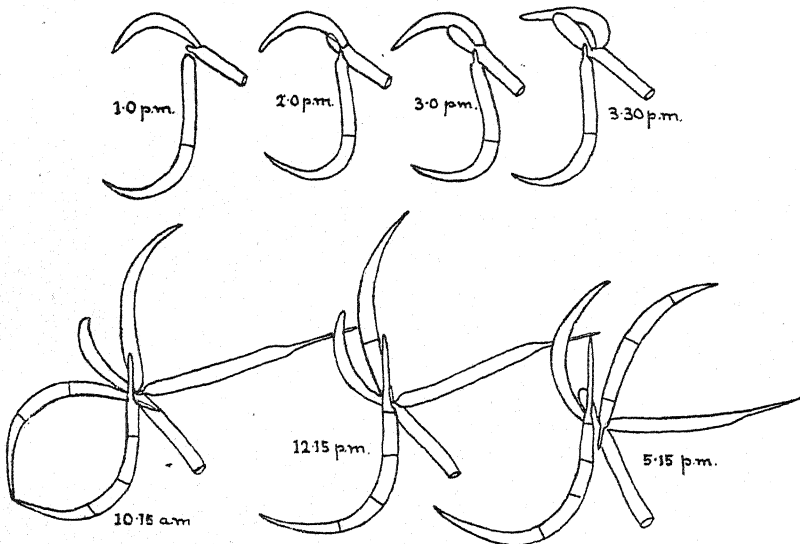


Fig. 5. The production of conidia in a hanging drop. The same conidiophore drawn at intervals on two successive days to show successive development of conidia ( $\times 850$ ).

curved, and narrowed towards the apex but not sharply pointed. The size at this stage is about  $25-30 \times 2.5-3.5\ \mu$ . Gradually the apex becomes more drawn out and at the same time from one to three septa appear, giving the *Cercospora* appearance which is that usually seen when diseased leaves are examined. After this the conidia fall away from the conidiophores and are no doubt soon washed away. In cultures in hanging drops of sterilised antirrhinum extract the whole development was followed more in detail, and is shown in Fig. 5. The first-formed conidium is pushed to one side by continued growth of the apex of the conidiophore and a second conidium is quickly produced. By the time the primordium of



a third conidium becomes visible between the two previously formed the first conidium has acquired its pointed apex and has begun to show the transverse septa. By continued growth of the third conidium it is gradually pushed more to one side and falls away, remaining (unless disturbed) lying free by the side of the parent conidiophore. Almost immediately after the conidium has become detached it is seen to have a small pedicel-like projection at its base. In the course of a few hours this projection elongates and finally becomes a more or less pointed appendage of about one-third the length of the spore at the time of detachment. Simultaneously the apex of the spore has become drawn out to a long, fine point, with the result that the final form of the spore, prior to germination, is as shown in Figs. 2, c and 5. The late development of the basal appendage

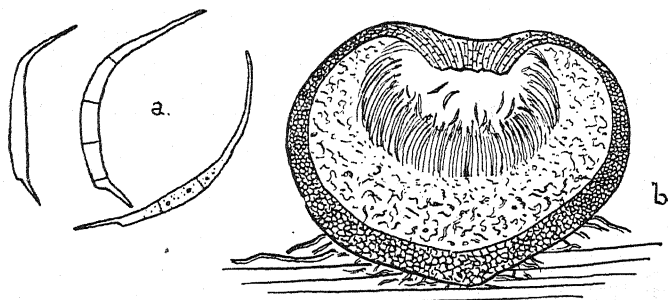


Fig. 6. *Heteropatella* stage. a, Conidia ( $\times 850$ ).  
b, Vertical section of young pycnidium.

is a point of morphological importance, because the similarly shaped spores of species of *Heteropatella* have by various authors been described as "pedicellate."

The development of the conidium from its first appearance as a small projection at the tip of a conidiophore until it is ready to be detached appears to occupy about seven hours at ordinary room temperatures. After the conidium is shed the development of the basal appendage and the elongated apex may occupy several hours more, but the times have not been exactly noted. The measurements of mature conidia are: body  $25-30(-35) \times 3.5-4 \mu$ , apical appendage about  $20-25 \mu$ , basal appendage up to  $10 \mu$  long.

The successive formation of conidia goes on for some time, and in an undisturbed hanging drop fairly large bunches of conidia may be found at the tips of the conidiophores. The slightest motion in the liquid, however, disperses them, and only the two or three conidia as yet immature remain attached.

(b) *Heteropatella* stage. The pycnidial form of the fungus has not been observed on leaves. On dead stems of antirrhinum it arises from the lignified tissue beneath the cortex, and eventually becomes superficial owing to the weathering away of the latter.

At first the pycnidium is a solid, somewhat flattened sclerotium-like body, consisting of a closely-woven mass of hyaline thick-walled hyphae, covered externally by a blackish pseudoparenchymatous cortex. Soon there appears near the outer surface a small flattened cavity, on the floor of which a dense layer of conidiophores is produced. It is now seen that the upper portion of the wall is thinner and different in structure from the basal part, in that the cells show a characteristic radial arrangement (Fig. 6, b). Later this lid-like outer wall is torn into segments from the centre outwards, the segments remaining as minute teeth fringing the margin of the flat conidia-bearing disc.

The conidia (Fig. 6, a) produced by this *Heteropatella* form of fructification are similar in shape and size to those of the *Cercospora*, and behave similarly on germination.

#### GERMINATION OF CONIDIA.

The germination of the conidia, whether those of the *Cercospora* or of the *Heteropatella*, and the subsequent early stages of development of the colonies, follow in general the course described by Brefeld(6) for the conidia of *Heterosphaeria Patella* and *H. Linariae* and by Vestergren(5) for *Heteropatella cercosperma*. In the media used by the authors, however (distilled water and sterilised antirrhinum extract), the production of secondary conidia immediately, without the formation of mycelium, has not been observed. Germination is always by means of a germ tube.

The cells of the conidium become much swollen, and at first a single germ tube arises laterally from one of the middle cells (Fig. 7, b, d). Later a second is formed towards the base of the conidium, and in forty-eight hours others may have been produced (Fig. 7, a, c, e). The basal appendage is often visible for a time (Fig. 7, e) but is soon obscured owing to the continued development and branching of the hyphae. Meanwhile the first-formed germ tubes have begun to produce conidia. As observed by Brefeld in the case of *Heterosphaeria Patella*, the first conidia are often different in form from the original sickle-shaped spores: they are smaller, aseptate, oblong-elliptical, and straight or only slightly curved, and blunt at each end. After the second or third day the primary conidia are replaced by others which are drawn out at the apex and more distinctly curved (Fig. 8, a),

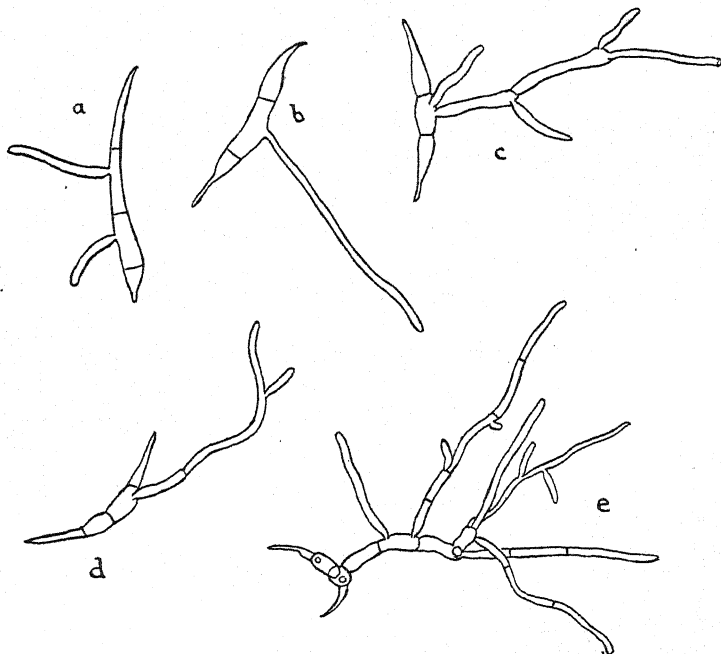


Fig. 7. Germination of conidia. *a, b, c*, Early stages, from hanging drop culture of *Cercospora* conidia. *d, e*, Later stages, from hanging drop culture of *Heteropateella* conidia.

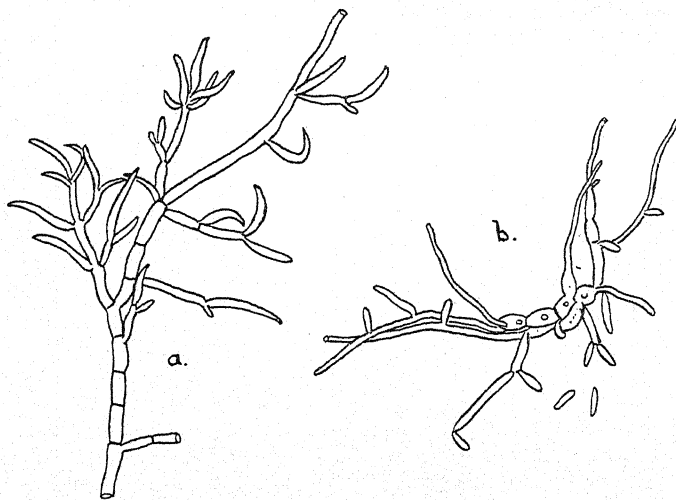


Fig. 8. Development of mycelium and conidia in hanging drop of sterilised antirrhinum extract. *a*, Normal conidia (early stage). (Culture from a *Cercospora* spore.) *b*, Showing development of simple cylindrical conidia in a medium which is drying out. (Culture from a *Heteropateella* spore.)

while on the fourth day the normal, septate, sickle-shaped conidia are present in great abundance, and when undisturbed remain hanging together in radiating clusters as already described. There appears to be a tendency for the primary form of conidium to be produced especially when the medium is drying out (Fig. 8, *b*).

#### TAXONOMY.

The discovery of a second "imperfect" fructification in the form of pycnidia belonging to the group Excipulaceae raises some interesting questions with regard to this fungus.

The first obvious comparison is with the so-called "winter conidial stage" which was described by Klebahn (7) in the case of *Entomopeziza Soraueri* and *Pseudopeziza Ribis*. In these two species the summer conidial form, in which the conidia are produced as in the Melanconiales, gives place on over-wintered leaves to a pycnidial fructification. Exactly the same type of conidium is produced, but the conidiophores are enclosed in a wall several layers of cells in thickness, at first closed but later widely open and more or less cup-shaped as in the Excipulaceae.

Klebahn later described a similar state of affairs in *Fabraea Fragariae* (8), where actually the winter conidia occur sometimes side by side with asci in an apothecium-like fruit body, and he compared this with the similar development which had been described by Tulasne (9) for *Heterosphaeria Patella*.

A further interesting point is to be noted in connection with *Pseudopeziza Ribis*. In the form of this species occurring on *Ribes Grossularia*, while the winter pycnidia are very freely produced, Klebahn was unable ever to induce the development of the ascigerous stage.

As already mentioned, the winter pycnidia of the antirrhinum fungus agree in character with the genus *Heteropatella*, species of which are known to be conidial stages of the Discomycete *Heterosphaeria*. Consequently an effort was made to find a perfect stage in this also. Up to the present, however, the search has been without success. Neither in culture nor on the old over-wintered Antirrhinum stems and leaves, even though kept under observation for some months, has there been any indication of the formation of asci.

It is, of course, possible that an ascigerous fructification does occur, and that the right conditions for its production have not been found. The various recorded observations on species of *Heterosphaeria* tend to show that the mature ascophore is formed only under specially favourable conditions. Thus Tulasne mentions that the perfect fruit bodies of *Heterosphaeria Patella* occur on the parts of stems nearest the soil, where the moisture

tends to be more constant. Fries held the view that the asco-phore (his form *a alpestre* of *Phacidium Patella*) occurs chiefly in subalpine regions because it requires a constantly damp atmosphere\*.

On the other hand, it is not unlikely that we have here a species which, in addition to developing the parasitic habit, has lost or is losing the power of developing asci. Klebahn discusses such a possibility in the case of *Fabraea Fragariae*, of which he says: "Nach den vorliegenden Beobachtungen macht *Fabraea Fragariae* den Eindruck eines Pilzes, der im Begriff steht, die Schlauchfruchtbildung zugunsten der Konidienbildung aufzugeben." Still more to the point is the case of *Gloeosporium Ribis* on *Ribes Grossularia* mentioned above, in which the *Pseudopeziza* stage has not been found, although it is known on other species of *Ribes*.

Comparison of the pycnidial stage of the antirrhinum fungus with described species of *Heteropatella* has shown that morphologically there is little or no distinction. All have conidia which average about  $18-25 \times 3-4 \mu$  without the appendages, and are from one- to three-septate. There are probably greater differences in the characters of the ascophores, but in the absence of the perfect stage it is difficult to say whether the species in question is new or can be referred to one of the others.

It is interesting to note, however, that there is a species, *Heterosphaeria Linariae* (Rabh.) Rehm, with its conidial stage *Heteropatella lacera* Fuck., which grows on the very closely allied host plant *Linaria vulgaris*. A careful comparison of the Antirrhinum *Heteropatella* with dried material of *H. lacera* has shown that morphologically they are very similar. Unfortunately fresh material of *H. lacera* has not been available, but fresh specimens of *Heterosphaeria Patella* on *Heracleum* were obtained at Tintern in April, 1925, and opportunity was taken to make pure cultures of this species. The growth in culture of *H. Patella* is quite different from that of the antirrhinum fungus, and the latter must therefore be regarded as distinct at least from the common species on Umbelliferous stems.

As there has been some confusion with regard to the taxonomy and nomenclature of the species of *Heterosphaeria*, it may be useful to summarise the facts as far as known.

The genus *Heterosphaeria* was proposed by Greville (10) in 1824, for a plant growing on dead herbaceous stems, chiefly of Umbelliferae, which had been called by Persoon *Sphaeria Patella*. Greville recognised that it was an Ascomycete, though

\* This may, however, be due merely to the fact that the species of *Heterosphaeria* in general grow best at low temperatures. See notes on temperature relations above.

not a *Sphaeria*, and described the "thecae," but was unable to find spores. Fries<sup>(11)</sup> in 1828 had apparently observed, but without understanding, the two stages of the fungus. In his *Elenchus Fungorum*, p. 133, he described two forms of what he called *Phacidium Patella*: the first, *a alpestre*, was described as having an apothecium with asci, while the second *β campestre*, was said to differ in remaining closed, and in never forming mature asci. He described the contents of the supposed closed fruit bodies of his *β campestre* as consisting of "sterile filiform asci."

It was left for Tulasne<sup>(12)</sup> to interpret the life history correctly, and to show that Fries's form *β campestre* was in reality the pycnidial stage, while his *Phacidium Patella, a alpestre* was the perfect stage of the fungus now known as *Heterosphaeria Patella* Grev.

Previous to Tulasne, Bonorden<sup>(13)</sup> in 1864 had given a full description of the pycnidial stage, referring it to Greville's *Heterosphaeria Patella*, and suggesting that Greville had been in error in thinking his plant an Ascomycete. Subsequently Hazslin<sup>(14)</sup> proposed the name *Excipula Bonordeni* for Bonorden's fungus, and this has been recently changed by Lind<sup>(15)</sup> to *Heteropatella Bonordeni*.

Meanwhile the genus *Heteropatella* had been proposed by Fuckel<sup>(16)</sup> in 1873, with the one species *H. lacera*, said to grow "on dead stems, chiefly of *Linaria vulgaris*." Fuckel supposed it to be "a Discomycete producing only conidia." The following year Winter<sup>(17)</sup> discovered asci in the *Linaria* fungus, and gave a description of this ascigerous stage under the name *Heteropatella lacera* (Fuck.) Wint. Rabenhorst later gave the name *Peziza Linariae* (changed by Rehm to *Heterosphaeria Linariae*) to this ascigerous stage, rightly reserving the generic name *Heteropatella* for the Excipulaceous conidial stage to which Fuckel's original description applied.

There seems to be little doubt that the species on *Linaria* and on Umbelliferae are distinct from one another. As indicated by Winter, both apothecia and ascospores are slightly smaller in *Heterosphaeria Linariae* than in *H. Patella*. It is obvious from Fuckel's description and from the specimens on *Linaria* distributed by him in his *Fungi Rhenani* no. 2565, that in the event of the two species being regarded as distinct, as they usually are, the name *Heteropatella lacera* should be applied only to the conidial stage of *Heterosphaeria Linariae* (Rabh.) Rehm. In spite of this however the name seems to have been used loosely even quite recently. Thus von Höhnelt (*Ann. Myc.* III (1905), p. 552 and *ibid.* XVI (1918), p. 35) speaks of *Heteropatella lacera* as the "young form of *Heterosphaeria Patella*," and Petrak (*Ann. Myc.* XIX (1921), p. 285) has fallen into the same error.

The best and clearest presentation of the common European species is that given by Lind in his *Danish Fungi* (15), where he has the following pairs of forms:

Ascigerous	Conidial
<i>Heterosphaeria Patella</i> Grev.	<i>Heteropatella Bonordeni</i> (Hazsl.) Lind.
<i>Heterosphaeria Linariae</i> (Rabh.) Rehm.	<i>Heteropatella lacera</i> Fuck.
<i>Heterosphaeria Patella</i> v. <i>alpestris</i> Fr.	<i>Heteropatella cercosperma</i> (Rostr.) Lind.

With regard to the third species, the conidial stage is that described by Vestergren in detail under the name *Rhabdospora cercosperma* (Rostr.) Sacc. It is obvious from the description and figures that the species is a *Heteropatella*. Vestergren recorded it as growing on numerous plants belonging to very different Natural Orders, in alpine and arctic regions, and for this geographical reason Lind has referred it to Fries's var. *alpestre* of *Heterosphaeria Patella*. It seems probable, however, that the name includes several species, and in view of the fact that Vestergren found temperature relations which agree with those of our *Antirrhinum* parasite, and may therefore be common to the whole group, it does not appear to be wise to take up the name *alpestris* with no further distinction. Obviously more exact work, with modern methods, is required as to the host relations and cultural characters of the *Heterosphaerias*.

In conclusion, reference must be made to the parasitic Hyphomycetous stage, *Cercosporella Antirrhini* Wakef., which was the starting point of the present investigation. In none of the known species of *Heterosphaeria* has such a stage been recorded. There is however a fungus which from the published descriptions is suggestively similar. This is a species on *Bupleurum* which was referred to *Heteropatella hendersonioides* Fautr. et Lamb. by Diedicke (18). Fautrey and Lambotte described their fungus on *Bupleurum falcatum* as having a pycnidium, and the spores as having three basal setae, Diedicke found his species on *Bupleurum longifolium*, and rightly or wrongly referred it to the same name. According to Diedicke, however, the structure of his plant is that of the Melanconiales, and not of a *Heteropatella*; that is, it has no pycnidial wall, but produces conidia in a layer covered only by the epidermis. Further, he was never able to see more than one basal appendage.

As every systematist knows, the distinction between the Melanconiales and the Hyphomycetes in certain species is difficult to draw, being a question of degree of development of the sporiferous layer. Diedicke's fungus, which he referred tentatively to *Pestalozzina* (19), is distinctly reminiscent of *Cercosporella Antirrhini*; it may possibly also have been para-

sitic, as it is said to occur "on extensive dry patches on leaves and stems."

It seems possible that the loss of a definite limiting pycnidial wall may be a new development in the life history of *Heterosphaeria* correlated with the adoption of a parasitic mode of life. One may perhaps go further and suggest that the antirrhinum parasite has arisen as an adaptation—a mutation even—from the species *Heterosphaeria Linariae* which occurs on the very closely allied host plant *Linaria vulgaris*, and that with the adoption of the parasitic mode of life it has lost the power to form asci and survives from season to season by means of the winter pycnidia.

Such a supposition would explain why an organism causing such conspicuous injury should yet apparently not have been described until so recently as 1918. It may be mentioned that one attempt has been made to inoculate *Linaria* with the antirrhinum fungus, but without result. It is hoped to do some further work in this direction.

#### SUMMARY.

The paper records observations on the life history of *Cercospora Antirrhini* Wakef., described for the first time in 1918 as a parasite of cultivated Antirrhinums.

In pure culture on certain media blackish apothecium-like bodies are produced, which however remain sterile unless transferred to fresh tubes, when they produce conidia.

Similar bodies were found on over-wintered dead stems of Antirrhinums which had shown the disease in the previous summer. These bodies were definitely pycnidial fructifications and were recognised as belonging to the genus *Heteropatella* Fuck.

The connection with the *Cercospora* was proved by inoculation of living Antirrhinum leaves with conidia from pure culture of the *Heteropatella*, when the characteristic leaf-spots were produced.

No ascigerous stage has as yet been found.

Relationship with *Heteropatella* is further confirmed by the temperature relations of the fungus, which agree with those found by Vestergren for a species identified as *Rhabdospora cercosperma* (Rostr.) Sacc. (= *Heteropatella cercosperma* (Rostr.) Lindl).

The optimum temperature lies at approximately 18° C., and no growth takes place at temperatures of 25° C. or higher. The conidia are killed by heating at 41° C. for five minutes in malt extract agar, and after one minute in prune agar.

A discussion of the taxonomy of the genera *Heteropatella* and



its ascigerous stage *Heterosphaeria* is appended, and it is suggested that the *Cercospora* may be a new development in the life history of *Heterosphaeria* correlated with the adoption of the parasitic habit. The antirrhinum fungus may have arisen as a saltation from *Heterosphaeria Linariae*, which occurs on dead stems of the closely related host plant *Linaria vulgaris*.

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## SUPPLEMENTARY NOTE.

By W. Buddin and E. M. Wakefield.

SINCE the foregoing paper was submitted for publication the authors have obtained living material of *Heteropatella lacera* Fuck. through the kindness of Mr A. S. Thomas, B.Sc., who collected it on old stems of *Linaria vulgaris* near Odiham, Hants, in the early spring of this year. Pure cultures of this species have been made, and compared as to growth characters and pathogenicity with those of the Antirrhinum parasite.

Morphologically there is very little distinction between the pycnidia of the two fungi. Those of *H. lacera* are larger ( $\frac{1}{2}$  to

1 mm. in diameter), and more decidedly black from the beginning than the small, brownish to black bodies which are found on old stems of *Antirrhinum*. The conidia of *H. lacera* have also perhaps slightly longer appendages, and are for the most part more strongly curved than those of the *Antirrhinum* fungus, but these differences are slight.

Cultural characters have given more definite information. Germination of the conidia in both cases is by means of one or more germ-tubes. In distilled water there is little difference, but with further growth on solid media, such as Dox's agar, distinct differences in development are to be observed between the two forms. In *H. lacera* from *Linaria* the short, cylindrical, budded-off conidia which were figured by Brefeld are formed very abundantly, so much so that a yeast-like mass is soon developed on the surface of the medium. The characteristic, mature, sickle-shaped conidia appear only at a rather late stage, and in relatively small numbers. In cultures of the *Antirrhinum* fungus, on the other hand, the short conidia are not formed in any great abundance except when the medium is drying out, whereas the sickle-shaped conidia appear within a few days and are the predominant form. Moreover there is, as a rule, more aerial mycelium developed in the case of the *Antirrhinum* fungus, so that cultures of the two fungi have from the first slightly different facies. In cultures of *H. lacera*, again, the blackish pycnidia begin to appear at an early stage, and in abundance, even on Dox's agar. As already described, those of *Cercospora Antirrhini* appear freely only in older cultures on organic media such as prune or malt agars.

The pycnidia of *H. lacera* as formed in culture are black, and when looked at with a lens appear slightly hairy externally. They remain for a long time closed. Those of *Cercospora Antirrhini* on the other hand are at first brownish in colour, quite smooth externally, and soon develop the open, discoid form. In neither are the marginal laciniae so conspicuous in culture as in nature.

Confirmation of the differences between the two fungi was obtained by means of inoculation experiments. Young plants of *Antirrhinum* were sprayed with spore-suspensions prepared from pure cultures of each form. Those sprayed with conidia from cultures of the *Heteropatella* from *Antirrhinum*s developed lesions on leaves and stems in about 14 days. Later the leaves showed the characteristic shot-hole effect, and many of the young green shoots were completely killed. Plants sprayed at the same time with conidia from pure cultures of the *Linaria* fungus remained perfectly healthy. The experiment was repeated with similar results. It may also be mentioned that an

attempt to inoculate *Linaria* in 1925 with conidia from the *Antirrhinum* form gave negative results.

There is therefore no doubt that the fungus causing shot-hole disease of *Antirrhinums* is distinct from the closely allied form on *Linaria vulgaris*. It differs in parasitism, and further differs from all other known species of *Heteropatella* in possessing a summer conidial stage which is morphologically a Hyphomycete. While this latter is obviously not really co-generic with other more typical *Cercosporellas*, there is at present no other form genus in which it could be better placed. In view of the occurrence of a definite pycnidial stage having the same type of spore, it will be preferable to adopt the name of the pycnidial stage than to create a new genus for the summer hyphomycetous stage. Since the pycnidial stage has been shown to be biologically and to some extent morphologically distinct from *Heteropatella lacera*, it should receive a new specific name, and may be called *Heteropatella Antirrhini* Buddin et Wakef.

In spite of further search no trace of an ascigerous stage has yet been found.

***Heteropatella Antirrhini*** Buddin et Wakef. sp. nov.

Pycnidia in ligno decorticato plantae nutricantis superficialia, sparsa, circa 500–600  $\mu$  diametro, e brunneo nigrescentia, globoso-depressa, primo clausa, deinde in lacinias irregulares dehiscentia. Substantia interna coriacea, albida, ex hyphis crasse tunicatis densissimeque intertextis composita. Discus conidiophorus planus, carnosus, pallidus. Conidiophora dense verticaliter disposita, filiformia, ramosa. Conidia falcata, utrinque attenuata, hyalina, 2–3-septata, 25–30(–35)  $\times$  3–4  $\mu$ , apice appendiculo filiformi, 20–25  $\mu$  longo praedita, basi propter appendiculum breviorum quasi pedicellata, in globulos pallide roseos emergentia.

*Hab.* In caulibus emortuis decorticatis *Antirrhini majoris*. Adest status hyphomycetalis parasiticus, *Cercospora Antirrhini* Wakef. foliis praecipue valde noxiis.

## CRYPTOTHECIACEAE.

## A FAMILY OF PRIMITIVE LICHENS.

(With Plate VII.)

By A. Lorrain Smith.

SOME years ago, as part of my study of British lichens, I had the privilege of looking over the late Dr Stirton's large collection of British and foreign specimens. Cordial thanks are due to the authorities in Glasgow, to whom the Stirton herbarium had been consigned, for giving me the opportunity to examine, and thus include in the *Monograph of the British Lichens*, the plants, new or old, determined by him. Dr Stirton was an indefatigable collector and worker with a wide knowledge of lichens: he has added several species to the British flora.

The greater number of specimens in his collection were, however, exotic, sent to him by friends from many lands—Canada, South and West Africa, New Zealand, Australia, India and elsewhere. Many of the plants had been determined, rightly or wrongly, by Stirton and include many new species. The greater number, however, enclosed in neat packets, bear a label inscribed only with the date, the name of the collector, the locality and, as a rule, the generic name. Pressure of other work prevented any study of the unnamed specimens, but records were kept of those with definite determination.

Among the more interesting were the plants labelled *Cryptothecia*, a puzzle to all lichenologists since the publication of a species, *Cryptothecia subnidulans*, by Stirton in *Proc. Phil. Soc. Glasgow*, x (1877), p. 164, as follows:

"*Cryptothecia subnidulans* sp. nov.

"Thallus albidus vel pallidus vel passim pallide cinereo-glauescens, nonnihil farinaceus, molliusculus saepe chryso-gonidicus, ambitu radiatim byssino-fibrillosus, bene evolutus (iodo sordide coerulescens, sed C-); apothecia nulla (propria sic dicta) visibilia, solum apicibus thecarum thallo obtectis vel nudis, protrudentibus vel prominulis (C. erythrinosi); thecae late ovatae monosporae rarissime 2-sporae in fibrillis anastomosantibus inclusae, sporae incolores oblongae interdum curvulae murali-divisae, episporio tenuisculo,  $0.05-0.075 \times 0.02-0.032$  mm., iodo flavescens. Iodo gel. hym. coerulescens vel (in speciminibus africanis) obsolete coerulescens vel haud tincta.

"Ad cortices arborum in montibus Nilgherrensibus (Dr. Watt) et prope Victoriam in Africa tropicali (Thomson).

"This lichen is allied to *Arthonia aleurodes* (Nyl.) and *A. sub-simillima*.

"The thecae are found pretty equally distributed throughout the thallus, and not congregated in groups so as to constitute true apothecia; accordingly, I have separated this and several others from the genus *Arthonia*."

The genus, as such, was placed on record by A. Zahlbruckner in Engler and Prantl, *Nat. Pflanzenf.* 1, 1\* (1903), p. 92, as "imperfectly known and of obscure position": his statement stands as the first publication of the genus.

After due consideration I have come to the conclusion that *Cryptothecia* is the representative of an entirely new family of lichens—primitive in the formation of the fructification, though advanced in spore characters, and, among lichens, most nearly associated with the Pyrenolichen families—Thelocarpaceae and Mycoporaceae. The nearest fungal allies are apparently the Myriangiales\* which *Cryptothecia* resembles in the solitary embedded asci without paraphyses. There is also undoubtedly a close affinity with the Plectascales, especially with Gymnoascaceae, in which family a similar loose peridium surrounds a fruiting body, differing in the numerous asci but also without paraphyses. The characters of these two groups in the case of the fungi suggest a relationship or a common ancestor. The difficulty of deciding the systematic position of the Myriangiales was appreciated by G. Arnaud who discusses the problem in his work on Les Astérinées (*Ann. Sci. Nat. Sér. 10, VII* (1925), p. 653. He decided finally that the Myriangiales were derived evidently from some type of primitive Ascomycetes: "One could," he states, "consider them as Plectascineae (for example Gymnoascaceae) in which the felted tissue which surrounds the asci would have become a compact tissue."

It was evidently the presence of the loosely interwoven peridium that led Stirton to suggest affinity with the Arthoniaceae in which there is a dense epithecium of branching paraphyses. The ascus of *Cryptothecia* with its peridium is a separate fruiting body each isolated in its covering of hyphae—though occasionally in groups—and deeply embedded during the whole development in the thalline tissue. It emerges readily on pressure when mature.

#### CRYPTOTHECIACEAE.

Thallus heteromerus, crustaceus, epiphloeodes, effusus interdum determinatus, non corticatus, hyphis medullaribus substrato adfixus. Gonidia palmellacea, vel stratum gonidiale

\* It is interesting to note that for a long time the genus *Myriangium* was classified as a lichen, and placed as the solitary member of a family Myriangiacei. (See Crombie, *Monogr. Brit. Lich.* 1, pp. 12 and 15, 1894.)

vel glomerulos discretos formantia. Fructificatio pyrenocarpea, in thallo immersa; perithecia solitaria, dispersa vel approximata; peridium ex hyphis ramosis, contextis, tenuibus formatum, ascum singulum (vel binum?) continens; paraphyses nullae; sporae 1-8nae murali-divisae vel septatae. Pycnidia non visa.

### Family CRYPTOTHECIACEAE.

Thallus crustaceous, epiphloeodal, superficial, or entangled with the outer cells of the bark without a cortex, rather thin, and compact. Algal cells, Palmellaceae (?) bright green, small, subglobose, about  $7-10\mu$  in diameter or subellipsoid. Reproductive body a perithecium containing one ascus, solitary, sparse or sometimes in groups, each with a peridium of colourless or brownish loosely-wetted hyphae, immersed in the thallus beneath the gonidial zone; paraphyses not formed; spores 1-8 in the ascus, colourless, muriform or septate.

The thallus, a sterile-seeming crust, recalls that of *Crocynia*, but is of denser texture. Stirton has described the fruit bodies as finally protruding from the thallus, an observation I have failed to verify, but the specimens, with one exception, were collected about fifty years ago and in several instances only empty asci, or stray spores have been found, though marked by Stirton as fertile. Stirton has recorded two asci in the perithecium: I have not been able to confirm that. The perithecia may be widely spaced or developed in close proximity.

The gonidia as far as seen are bright green, no chrysogonidia (*Trentepohlia*) as recorded by Stirton have been observed.

The lichen may evidently spread to two or three inches or more and in some instances it covers other crustaceous forms thus indicating a quick vigorous development; it grows on the uneven surface of the bark or round twigs, and occurs sometimes in quite small patches. The thallus is mostly very light-coloured sometimes with greenish patches where the gonidia have come near to the surface and sometimes darker at the edges. It is frequently covered with a fine furfur of minute cells budded off from the slender hyphae and the medulla may also be interspersed with similar cells; occasionally the ends of the hyphae project from the cortex. When water is applied it runs off in most of the specimens leaving the non-gelatinous surface dry.

It is generally impossible to determine before making sections the position of the fruit bodies in any specimen, though they tend to develop in the thicker portions, or in small wart-like nodules and pustules when these are present.

The specimens from India were collected by Sir G. Watt; those from West Africa by Mr G. Thomson.

Two genera, differing in spore characters, are represented among Stirton's specimens:

Spores muriform	.	1. <i>Cryptothecia</i> .
Spores septate	.	2. <i>Stirtonia</i> .

1. *Cryptothecia* Stirton apud A. Zahlbr. *loc. cit.*

Crusta effusa, sat tenuis, subdeterminata, non corticata. Gonidia minuta, in stratum continuum vel glomeratum disposita. Perithecia infra stratum gonidiale immersa; asci solitarii; sporae 1-8nae, incolores, murali-divisae.

In the genus *Cryptothecia* the thallus is spreading, smooth or finely furfuraceous or slightly uneven with small nodules or wrinkles, whitish or greyish, effuse or sometimes white fimbriate at the margins and determinate, about  $100\mu$  to  $250\mu$  thick; upper cortical tissue intricate, composed of slender closely-vefted hyphae, and about  $30\mu$  from the surface to the gonidial zone; gonidia in an irregular loose layer varying in depth to about  $40\mu$  and subcontinuous or occasionally in patches, the medulla of rather densely entangled hyphae similar to those of the cortical layer adhering closely to the substratum and entangled with the cells of the outer bark. Perithecia ovoid, pyriform or almost spherical, entirely immersed below the gonidial zone, the peridium of vefted hyphae about  $5-10\mu$  thick; ascus almost as large as the perithecium, generally thick walled over the apex; spores 1-8 in the ascus, colourless, broadly oblong or ovoid, muriform.

Ascus 1-spored.

1. *C. subnidulans* (Stirton, *loc. cit.* descr. generica nulla) A.L.Sm.

Thallus cinereus vel albidus effusus, uniformis, laevigatus vel verrucis minutis obsitus, tenuis, usque  $200\mu$  altus, margine fimbriato albido cinctus (K-, CaCl-, vel erythrinosa). Perithecia subpyriformia, usque  $100\mu$  long.  $55\mu$  lat.; sporae 1-nae, hyalinae, multi-septatae et murali-divisae  $50-60\mu$  long.,  $25-30\mu$  cr., cellulis parvis, numerosis.

*Distr.* West Africa (Victoria, Cameroons); South-east Africa (Natal); India, Bengal (Chinsurah), Madras (Nilghirri Hills). Pl. VII, figs. 1, 2, 3.

The thallus is smooth or minutely nodulose, whitish or light cinereous-grey, subdeterminate and slightly white-fimbriate at the margins, rather widely spreading, usually about  $150\mu$  in depth (K -, CaCl + rose-red ov. -). Perithecia developed beneath the gonidia, easily emerging from the peridium when mature, the peridium about  $5-10\mu$  thick, becoming brownish; the single ascus pyriform, thick walled (about  $10\mu$  thick), very

variable in size from 60 to 100  $\mu$  long and from 45 to 55  $\mu$  wide; the spores, one in the ascus, up to 14 or more septate and muriform, variable in form but generally broadly oblong, sometimes curved, varying also in size from 50 to 65  $\mu$  long, 25 to 30  $\mu$  thick.

On testing with potash there is sometimes a faint yellow reaction which soon disappears. The rose-red colour on the application of calcium hypochlorite is also somewhat impermanent or not present.

The specimen recorded from South-east Africa differs slightly though not specifically; it was sent to me some years ago by Professor Van der Byl of Berea, Natal. It grew on the bark of *Albizzia fastigiata*; the thallus is lighter in colour and thicker than in the specimens from more tropical lands, reaching a depth of  $\frac{1}{2}$  mm. or even more. The tissue is somewhat loose, the hyphae appreciably stouter and the gonidia more scattered, 8–10  $\mu$  diam. and not easy to determine, though evidently Palmellaceae. These characters possibly indicate a moister habitat. The reaction with CaCl is + yellow then reddish, but slow to appear, and impermanent; the colour was obtained by using Magnusson's method of introducing a grain of bleaching powder into the water-mount. The periderm is rather thin, the ascus about 70  $\mu \times$  45  $\mu$  and the spore variable in size, 45–60  $\mu$  long, 15–22  $\mu$  thick; and irregularly muriform. Periderm I+ scarcely blue, ascus and spore red.

Ascus up to 8-spored.

## 2. *C. Stirtonii* A.L.Sm.

Thallus crustaceus, albidus vel cinereus, effusus subdeterminatus, margine interdum byssino-fibrillosus. Perithecia numerosa, peridio fibrilloso inclusa. Asci solitarii; sporae 8-nae, oblongae hyalinae, ca. 10-septatae et murali-divisae, 70–100  $\mu$  long., 35–50  $\mu$  lat., cellulis parvis numerosis. Pl. VII, fig. 4.

*Distr.* West Africa (Cameroons); Burmah (Rangoon); India (Tonglo, Himalayas 10,000 ft.), and Bengal (Chinsurah).

The thallus of *C. Stirtonii* scarcely differs from that of *C. subnidulans*: it is whitish, grey or greenish-cinereous, effuse or subdeterminate and thinly white-fimbriate at the margins, smooth or with occasional pustules (K – , CaCl – , I + blue in part). Perithecia situated beneath the gonidial zone, subpyriform or almost spherical, varying in size, but rather large: the peridium becoming brownish about 10  $\mu$  thick; ascus with a wall 10  $\mu$  thick or more and varying in size up to 130  $\mu$  long and 80  $\mu$  wide, or, if spherical, about 120  $\mu$  diam.; spores 8 in the ascus, large and broadly oblong, sometimes curved, but straight when



properly mature, also varying in size from  $70\ \mu$  long and  $35\ \mu$  thick to  $100\ \mu$  long and  $50\ \mu$  thick, about 10 or more septate and muriform, with 6 or more longitudinal divisions, the cells small and numerous.

The different specimens had been labelled as *Cryptothecia* and in some instances spore characters were added. I have named it in honour of Dr Stirton

3. *C. subtecta* (Stirton MS.) A.L.Sm.

Thallus albidus, cinereus, vel fusco-cinereus, laevigatus vel leviter purpureus, subdeterminatus (K — vel flavescens, CaCl —). Perithecia immersa, vel sparsa vel crebra, peridio hyphis fusciscentibus composito, ovoidea  $70$  ad  $100\ \mu$  long.  $50$ – $70\ \mu$  lat.; asci peritheciis subaequantur, membrana sat tenuis; sporae 8-nae, oblongae, 14-septatae et irregulariter murali-divisae, usque  $50$ – $40\ \mu$  long.  $15$ – $20\ \mu$  cr., cellulis parvis, numerosis. Pl. VII, fig. 5.

*Distr.* India, Bengal (Chinsurah) and Assam.

The thallus of *C. subtecta* is about  $150$  to  $200\ \mu$  in depth; the algal cells about  $7\ \mu$  diam. at times almost reaching the surface. Perithecia solitary or rather closely grouped, pyriform-ovoid from  $70$ – $100\ \mu$  long,  $50$ – $70\ \mu$  in width, the peridium brown in thick section; asci almost filling the perithecium, with a thin membrane about  $5\ \mu$  in width; spores broadly oblong, irregularly muriform; about 14-septate and 5 to 6 longitudinal divisions,  $30$ – $40\ \mu$  long,  $15$ – $20\ \mu$  thick.

The specimens were labelled by Stirton as *C. subtecta*; the perithecia are completely hidden in the thallus without any indication of their presence. It differs from *C. Stirtonii* in form and size of perithecia, spores, etc. There is occasionally a slight tinge of yellow on the application of potash; the reaction with iodine is variable, the perithecia and parts of the thallus are occasionally tinged blue.

4. *C. conferta* A.L.Sm.

Thallus tenuis, albido-cinereus vel virescens, laevigatus vel leviter verruculosus, subdeterminatus et margine fimbriatus (K —, CaCl —). Perithecia normaliter ad basim inter sese conferta sed distincta, ovoideo-elongata usque  $80\ \mu$  long.  $45\ \mu$  lat. Peridium hyphis peridiis fusciscentibus compositum; asci membrana tenuis sed apice crassior usque ad  $10\ \mu$  lat., sporae 8-nae, ovoideae, 6–9-septatae et irregulariter murali-divisae, ca.  $30\ \mu$  long.  $17\ \mu$  cr., cellulis parvis, numerosis. Pl. VII, fig. 6.

*Distr.* India, Assam.

The thallus is thin, about  $140\ \mu$  thick, greenish or greyish white, smooth or dotted with minute nodules, subdeterminate and indistinctly fimbriate at the margins. Algal cells are  $7$ – $10\ \mu$  diam.

in a gonidial zone or in dense groups near the surface sometimes giving a greenish appearance to the thallus. Perithecia with a brownish peridium, elongate-ovoid, about  $85\mu$  long,  $40\mu$  wide occurring in close groups attached at the base; the ascus with a narrow wall widening over the tip to about  $10\mu$ ; spores 8 in the ascus, elongate-ovate, with a clear gelatinous wall, irregularly muriform, 6-9-septate with 2-4 longitudinal divisions  $25-30\mu$  long,  $10-17\mu$  thick. *C. conferta* marks a distinct association of the perithecia, a character somewhat suggesting the pseudo-stromata of Mycoporaceae. It differs from other species in the closely approximate perithecia and in the smaller spores.

2. *Stirtonia* A.L.Sm. gen.nov.

Thallus crustaceus, effusus. Algae Palmellaceae minutae. Perithecia in thallo immersa, peridio e hyphis contextis composito, ascum singulum continens; sporae 8-nae, hyalinae, pluri-septatae.

The thallus does not materially differ from that of *Cryptothecia*. I have pleasure in naming the genus after Dr Stirton.

1. *St. obvallata* A.L.Sm.

Thallus sat parvus, cinereo-albidus, fibrillae marginis paulum distinctae (K —, CaCl —, I + caerulescens). Stratum gonidiale non bene distinctum, gonidia parva ca.  $7\mu$  diam. Perithecia immersa; asci fere ovoidei,  $90-110\mu$  long.,  $55-70\mu$  lat. Sporae oblongae, rectae vel leviter curvulae, membranis cinctae, 6-8-septatae,  $50-90\mu$  long.  $20-30\mu$  cr. Pl. VII, fig. 7.

*Distr.* India, Bengal (Chinsurah).

*Stirtonia obvallata* is distinguished by the thin thallus with a very distinct reaction with iodine; the gonidial layer is also a thin layer of small scattered algae about  $7\mu$  diam. and delicate intricate hyphae. The perithecia are deeply immersed, the peridium also becoming blue with iodine; the asci somewhat ovoid  $90-110\mu$  long,  $55-70\mu$  wide; the spores broadly oblong sometimes slightly curved with a clear epispore, very distinctly and mostly 7-septate,  $60-90\mu$  long,  $20-30\mu$  thick.

The species was labelled by Stirton as *Cryptothecia obvallata* but not published. It was collected by D. G. Watt.

2. *St. dubia* A.L.Sm.

Thallus effusus, pertenuis, albido-cinereus, leviter furfuraceus (K —, CaCl —, I + caerulescens). Gonidia minuta, ca.  $5\mu$  diam. Perithecia immersa, peridio e hyphis contextis composito; ascus ovoideus, usque  $55\mu$  long.  $30\mu$  lat.; sporae hyalinae fusiformes, elongatae, rectae, normaliter 7-septatae,  $25-30\mu$  long.  $7\mu$  cr., I + vinose rubens. Pl. VII, fig. 8.

*Distr.* India, Bengal (Chinsurah).

There is only one specimen of this lichen which is growing on wood and is bordered by a dark line possibly due to an adjoining species. The thallus is extremely thin and the different tissue layers difficult to determine; there is a distinct blue reaction with iodine, especially in the neighbourhood of the perithecia. The algal cells are small and scanty. The peridium enclosing the perithecium is rather thin; the spores are distinctly septate without longitudinal divisions, elongate-fusiform  $25-30\ \mu \times 7\ \mu$  with the middle cells slightly larger. It is possible that they might be immature and ultimately become more or less muriform but the form of the spores as well as of the septation agrees with *Stirtonia*.

I am indebted to Mr E. H. Ellis for the photomicrographs and assistance with the drawings.

#### EXPLANATION OF PLATE VII.

- Fig. 1. Section through thallus of *Cryptothecia subnidulans* showing ( $\times 45$ ): a, bark of tree; b, immersed perithecia; c, position of gonidial layer; d, cortex.  
 Fig. 2. *C. subnidulans*: ruptured peridium and ascus with spores.  $\times 180$ .  
 Fig. 3. *C. subnidulans*: spore.  $\times 440$ .  
 Fig. 4. *C. Stirtonii*: ruptured peridium and ascus with spores.  $\times 125$ .  
 Fig. 5. *C. subsecta*: spore.  $\times 440$ .  
 Fig. 6. *C. conferta*: ascus with spores.  $\times 440$ .  
 Fig. 7. *Stirtonia obvallata*: ascus with spores.  $\times 440$ .  
 Fig. 8. *S. dubia*: ascus with spores.  $\times 440$ .

### THE GENUS *LIGNIERA* MAIRE & TISON.

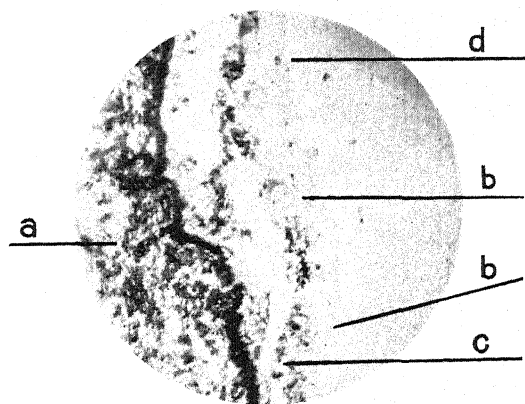
(With Plates VIII and IX.)

By W. R. Ivimey Cook, B.Sc.

#### INTRODUCTION.

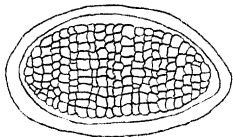
In the genus *Ligniera* have been placed those Plasmodiophoraceous fungi which cause no, or practically no hypertrophy of the host tissues. They occur solely in the roots of Phanerogams.

In 1910 Blomfield and Schwartz (1) described and figured the life history and cytology of *Sorosphaera Veronicae* which caused hypertrophy of the stems of *Veronica Chamaedrys*. Later in the same year and again in 1911 Schwartz (20, 21), described two further species, *Sorosphaera Junci* and *S. graminis* occurring in the roots of *Juncus articulatus* and *Poa annua* respectively. Maire and Tison in 1911 (11, 12), constituted the genus *Ligniera* to include two species which they had themselves discovered, *L. verrucosa* in the roots of *Veronica arvensis*, and *L. radicalis* in those of *Callitriche stagnalis*. They also transferred *Sorosphaera Junci* to their new genus *Ligniera* on the ground that unlike *S. Veronicae* it caused no hypertrophy of the host tissues. The following year Winge (23), reviewing the Plasmodiophorales, included in the genus *Ligniera* the three

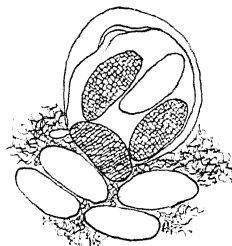


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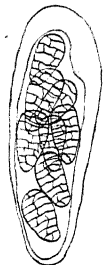
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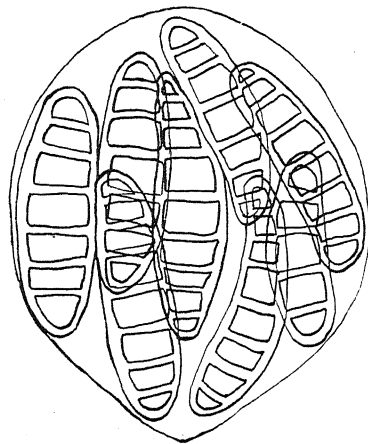
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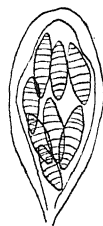
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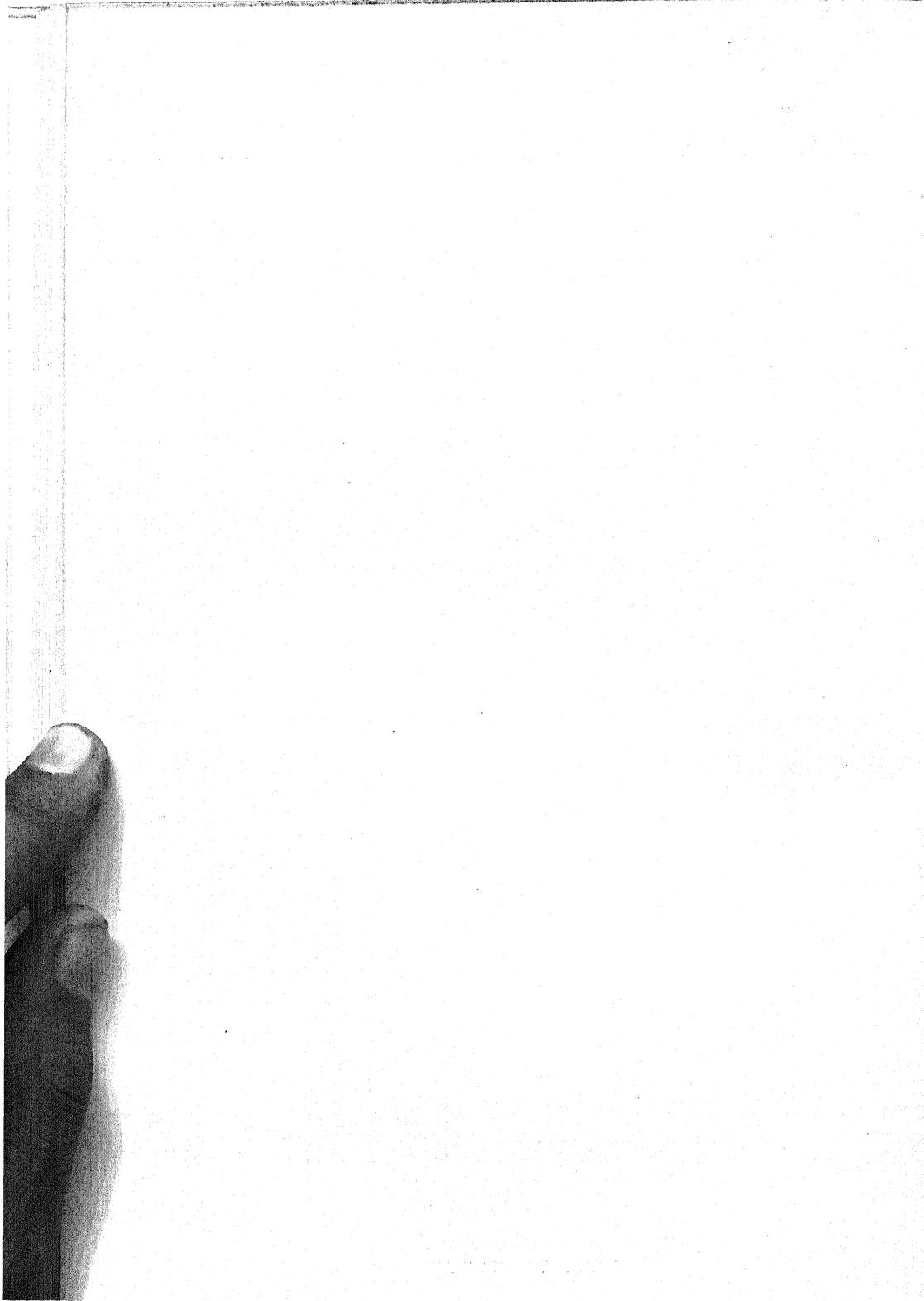
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species already described and also added *Sorosphaera graminis* the life history of which had been published before Maire and Tison's paper had been received in this country. In 1914 Schwartz<sup>(22)</sup> adopted these alterations and briefly described three more species of *Ligniera* from the same locality where he had obtained *L. Junci* and *L. graminis*, and named them after their host plants *L. Bellidis*, *L. Menthae* and *L. Alismatis*.

In 1925 Fron and Gaillat<sup>(6)</sup> described from France a species of *Ligniera* occurring in the root hairs of *Poa annua*, which they called *L. pilorum*. They separated *L. pilorum* from *L. radialis* on the ground that their species caused some hypertrophy of the root hairs. They did not, however, cite Schwartz's paper on *Sorosphaera graminis* in which the fungus is recorded as attacking the roots of *Poa annua*.

In the summer of 1924 material of *Ranunculus circinatus* infected with a species of *Ligniera* was given to me by Dr E. J. Schwartz. Unfortunately the roots were not sufficiently well preserved to enable me to make out any cytological detail. This material had been collected from the same locality as the other species of *Ligniera* which he had previously described. In the autumn of 1924 a visit was made to the pond where this infected *Ranunculus circinatus* was growing, and a good supply of roots was collected and fixed. At the same time roots of other associated water plants were collected.

On microscopic examination it was found that these roots were all heavily infected with a species of *Ligniera*. Since all the plants were growing in the same pond under conditions entirely suitable for infection from one another, it was thought extremely likely that all the roots were diseased with the same species of *Ligniera*.

About the same time roots of *Plantago major* growing in association with diseased roots of *Poa annua* were found to be infected with *Ligniera*.

From a consideration of the above facts together with the very close microscopic resemblance, doubt was cast upon the validity of the five species of *Ligniera* already described from the neighbourhood of Sevenoaks.

The following spring suitable plants were grown in the greenhouses at King's College, London, under conditions which rendered infection from *Ligniera* impossible, and after they had passed the seedling stage they were inoculated with roots heavily infected with suitable species of *Ligniera*.

At the same time, having a considerable quantity of diseased roots at my disposal, a microscopic examination was made in the hope of elucidating some cytological points which had not been very clearly described by previous workers.

Many roots showed stages in the formation of zoospores; these were recorded by Maire and Tison in *Ligniera radicalis* but were not observed by Schwartz in any of his material. The former workers, however, did not follow the subsequent fate of these zoospores.

Using living material, a study of the mode of infection by zoospores was made.

#### INOCULATIONS.

In the early spring of 1925 seeds of the following plants were sown in sterile soil which had been autoclaved at a temperature of 130° C.: *Plantago major*, *Poa annua*, *Mentha Pulegium*, *Iris Pseudacorus*, *Veronica Beccabunga* and *Ranunculus circinatus*. The seedlings were potted forward, and by the end of May were strong healthy plants.

During the spring more examinations were carried out on the water plants growing in Knole Park, Sevenoaks, with the result that *Ligniera* was found to be present in *Ranunculus circinatus*, *Potamogeton natans*, *Iris Pseudacorus*, *Juncus articulatus*, *Ranunculus aquatilis* and *Callitriche stagnalis*.

From marshy ground in the same locality *Ligniera* was found in *Bellis perennis*, *Plantago major* and *Poa annua*; and in some ditches near by in *Alisma Plantago-aquatica*.

Near a large pond on Chislehurst Common, Kent, infected plants of *Mentha Pulegium* were also collected.

The infection of seedlings was carried out during May, 1925, and the experiments were divided into two groups, firstly the infection of healthy plants by diseased roots of the same host; and secondly by infecting plants with the disease by using the roots of a different host. Plants to be inoculated were knocked out of their pots and their roots broken and damaged; roots, also bruised and heavily infected with the disease were then placed in close association with them, and the whole repotted and kept well watered. In no case was the health of the host plant affected by this treatment, and in the great majority of cases the roots of the infecting plant also grew.

Towards the end of September all the plants were lifted and their roots examined for the presence of any fungus. In each series of experiments a certain number of plants, usually five, were kept as controls. The following table shows the number of plants which were infected and the results obtained.

The first column shows the number of plants inoculated and the second the number which became diseased.

From Table I it will be seen that *Ligniera* from the roots of seven different hosts was used for infection.

Out of thirty-two plants infected with the parasite from *Poa annua*, thirty-one developed the disease. No attempts were made to infect either *Mentha Pulegium* or *Veronica Beccabunga* owing to the small number of plants at my disposal. Diseased roots of *Plantago major* transmitted the disease to the roots of *Poa annua*, *Mentha Pulegium*, *Ranunculus circinatus*, *Iris Pseudacorus* and to healthy plants of the same species; out of twenty-eight plants twenty-six became infected.

Table I. *Plants inoculated.*

Material used in inoculation	<i>Plantago major</i>		<i>Poa annua</i>		<i>Mentha Pulegium</i>		<i>Ranun- culus circinatus</i>		<i>Iris Pseud- acorus</i>		<i>Veronica Becca- bunga</i>	
<i>Poa annua</i> *	10	10	5	5	—	—	12	11	5	5	—	—
<i>Plantago major</i> *	3	3	5	4	5	5	5	4	10	10	—	—
<i>Ranunculus aquatilis</i> †	12	10	5	4	3	3	—	—	10	9	—	—
<i>Mentha Pulegium</i> ‡	5	5	—	—	—	—	5	5	5	4	—	—
<i>Callitriche stagnalis</i> †	10	10	10	10	5	4	5	4	10	8	—	—
<i>Veronica Beccabunga</i> §	—	—	5	5	—	—	—	—	—	—	5	4
<i>Juncus articulatus</i> †	—	—	—	—	—	—	5	4	—	—	5	5
Controls (five plants)	0	0	0	0	0	0	0	0	0	0	0	0
Total plants	40	38	30	28	13	12	32	28	40	36	10	9

The plants were collected from the following localities:

\* Marshy ground near Sevenoaks; † pond, Sevenoaks; ‡ Chislehurst; § Cambridge.

Heavily infected roots of *Ranunculus aquatilis* were used to inoculate thirty plants, and twenty-six of these became diseased. Only fifteen diseased plants of *Mentha Pulegium* were available for use as inoculants and five plants each of *Plantago major*, *Ranunculus hederaceus* and *Iris Pseudacorus* were used. As a result all except one plant developed the disease.

A large quantity of suitably infected material of *Callitriche stagnalis* was available and was used for experiments with *Plantago major*, *Poa annua*, *Mentha Pulegium*, *Ranunculus circinatus* and *Iris Pseudacorus*; out of forty plants thirty-six became infected.

*Veronica Beccabunga*, owing to its habitat, was not convenient to use for infecting other plants, and it was only tried with *Poa annua*; five plants were inoculated and all became diseased. Healthy plants of *Veronica Beccabunga* growing with diseased ones developed the fungus.

Good material of *Juncus articulatus* was difficult to obtain; five plants each of *Ranunculus circinatus* and *Veronica Beccabunga* were inoculated with diseased *Juncus* and all except one became infected.

In every case five control plants of each species were grown through the summer in sterile soil without being inoculated, and when examined in the autumn were all found to be free from *Ligniera*.

In all out of 165 plants used in the inoculation experiments, 151 had developed the disease after four months. That a few



should remain free from any infection is not surprising considering the methods used. As it was not possible to examine microscopically every root used as an inoculant, there was always the possibility that a few were free from any disease; this alone is sufficient to account for failure in the fourteen instances.

I was unable to carry out any satisfactory experiments with *Alisma Plantago-aquatica* owing to difficulty in keeping the plants alive in the greenhouse. Only one specimen was available for inoculation and it was grown in association with *Poa annua*. Its roots on examination later were found to be infected with *Ligniera*.

From the above results there is no doubt that, in the absence of any cytological distinctions, *Ligniera Junci*, *L. graminis*, *L. Bellidis*, *L. Menthae* and *L. Alismatis* are all the same species, which is capable, under suitable conditions, of infecting the roots of many Phanerogams growing in aquatic or marshy habitats.

During the summer of 1925 *Ligniera* was also found in the roots of *Galium verum* and *Ranunculus aquatilis* from Sevenoaks, in *Veronica Beccabunga* from Tintern, Mon., and in *Cerastium vulgatum* from Westerham, Surrey.

It is interesting to note that the fungus does not attack the roots of certain plants even when these are growing in close association with heavily infected roots of other species. A particular case which was brought to my notice was that of *Valeriana dioica* the roots of which were totally free from the disease, although plants of *Poa annua* and *Bellis perennis* growing close by were infected. The reason for this is not clear, but it may be connected with the relative acidity of the soil or to the presence of some substance in the roots themselves. Schwartz<sup>(20)</sup> pointed out that all the forms of *Ligniera* which he found, appeared in plants growing in marshy meadows, in which were springs breaking out at the junction of the Folkestone beds (Lower Greensand) with the Gault. In such places the soil is rich and heavily charged with iron salts. He also noticed that *Sorosphaera Veronicæ* occurred much more frequently in such localities. *Valeriana dioica* is a chalk-loving plant and this difference in soil and situation may be an important factor in the infection of a host. In general, both *Ligniera* and *Sorosphaera* are found most commonly in rich alluvial soil containing an excess of iron. Experiments regarding the composition of the soil of infected plants are now in progress.

## MICROSCOPIC EXAMINATION.

### *Methods.*

A considerable quantity of suitably infected material was at my disposal as a result of the above inoculation experiments.

For fixation, Flemming's weaker solution, chrome acetic, Bouin's fluid, and Allen's modification of Bouin were used. Several corrosive sublimate formulae were also tried. These latter were not very satisfactory, tending rather to harden the roots. With alcoholic corrosive sublimate and picric acid considerable plasmolysis of the cells took place. Acetic-alcohol also proved too vigorous a fixative to be used. The most perfect fixation was obtained with Flemming's weaker solution and Bouin's fluid. No advantage was found in using Allen's modification, and eventually only Bouin's fluid was used. Large masses of fine roots were put in this fixative and after being air pumped were left for twenty-four hours. They were then washed in water and graded into 75 per cent. alcohol.

In selecting roots for examination very little notice was taken of the particular host plant used. Attention was chiefly paid to selecting those free from adhering detritus or any bacterial infection.

Two complete series of slides were made, one series stained in cotton blue and the other in Haidenhain's haematoxylin.

Small pieces of fixed root were examined microscopically and if heavily infected were put aside in 75 per cent. alcohol. In preparing cotton blue mounts the following method was found most satisfactory.

A solution of lacto-phenol, prepared by mixing one part each of lactic acid, glycerine, phenol and water was used as the mounting fluid. For staining a 0.3 per cent. solution of cotton blue was made up in lacto-phenol. Complete roots, a centimetre or so long, were removed from the alcohol and the spirit allowed to evaporate for a minute, a drop of lacto-phenol was then added and the slide was warmed until the fluid just began to steam. The lacto-phenol was drained off and replaced by a drop of the stain. The slide was warmed as before, and the cotton blue was allowed to act for twenty-five seconds. It was then drained off and the excess of stain washed off with lacto-phenol. When no more stain would come out the root was carefully rolled with a round pencil. In this way not only was the root flattened, but all excess of stain still in the vessels was squeezed out. The root was then placed on a clean slide further teased out with needles and mounted in a drop of lacto-phenol. Slides

were ringed with two coats of gum dammar in xylol, and then with one of black enamel.

In the case of the iron alum haematoxylin preparations the normal method was employed. The roots were left in 4 per cent. iron alum over night and stained in Haidenhain's haematoxylin for two hours. The excess of stain was removed with acid alcohol, and here again it was found that rolling the roots greatly helped the removal of excess of stain. The roots were carefully teased out before mounting in balsam.

Several attempts were made to section the roots. Material was brought up into wax rapidly in twenty-four hours, and also slowly; wax of different melting points was tried; but in every case the results were unsatisfactory, sections cut at all thicknesses from  $2\mu$  to  $10\mu$  showing the same broken appearance. I am informed by Dr E. J. Schwartz that he found the same difficulty in the preparation of sections.

The results obtained from squashed roots were so satisfactory, however, that it was found quite unnecessary to use sections even for the examination of nuclear divisions.

#### LIFE HISTORY.

The earliest stage which is usually recognisable in the roots of an infected plant is a small spindle-shaped uninucleated amoeba (Plate VIII, fig. 1). This consists of a granular mass of protoplasm with a large central nucleus having a large karyosome, which can be clearly seen in the photograph. The amoeba is naked.

Nuclear division takes place very soon, and is accompanied by increase in size of the amoeba which frequently sends out pseudopodia from the ends of the long axis (Plate VIII, fig. 2 and Plate IX, fig. 2).

This nuclear division has been called the "cruciform division" and is really a type of protomitosis, similar in many respects to that described in several of the Protozoa belonging to the Order Lobosa. It seems probable that the divisions are completed very quickly, as stages are very difficult to find. As a result of this previous workers have not given a very satisfactory account of the stages. It is not proposed in this paper to give a full account of what happens, as there are still a number of points which have to be cleared up before the complete account can be published. As far as the work has progressed, the process conforms almost exactly with that given by Nawaschin (14) in *Plasmodiophora*.

At the time of nuclear division the chromatin becomes formed into a plate with the karyosome, which may be occasionally double, situated at the centre (Plate IX, fig. 3). In polar view nuclei

at this stage are very difficult to distinguish from resting nuclei. There is next a gradual aggregation of the chromatin towards the periphery of the plate forming what, in side view, appears to be a dumb-bell. The plate then splits longitudinally and the two halves travel apart, the karyosome becoming drawn out in the centre. Gradually the karyosome also splits and travels with the chromatin to the poles, where the chromatin becomes curved round the outer periphery of the karyosome at each end of the spindle. During the whole process the nuclear membrane remains intact, though during the later stages it becomes more drawn out, and finally, after the reconstitution of the daughter nuclei, becomes invaginated at the centre and finally breaks in half forming the nuclear membranes of the daughter nuclei. There are, especially during the early stages, very clear signs of a spindle which is equal on both sides of the plate and not on one side only as was described by Nawaschin. Further work on this division is in progress.

Whether divisions are frequent is not known, but as very few are met with it is concluded that they are passed through very rapidly as the amoeba grows in size. This growth continues at the expense of the host tissue but normally there is no hypertrophy of the host cell. When, however, the amoeba is lying in a root hair a swelling is sometimes noticed, though infected root hairs which show no hypertrophy are more common.

In some cases, as was observed by Schwartz (22), two uninucleated amoebae fuse to form a single binucleated amoeba. There is no evidence to show that this fusion is in any way a conjugation, though it occurs only between very young amoebae.

The full grown amoeba may be compared with the plasmodium of the Mycetozoa, which it resembles very closely. The size of the full-grown amoeba is usually limited by the size of the host cell, though if this cell is very large the contents may become exhausted before the amoeba has completely filled it.

About this time further changes are observed in the nucleus. The karyosome which up to now has been the most characteristic feature of the nucleus, disappears and the amoeba is seen with chromatin only around the nuclear membrane. Osborn (16) working on *Spongospora subterranea* considered that the nuclei are reconstituted on fresh sites. As far as my observations go there seems no reason for making such a statement concerning *Ligniera*. No example has so far been observed in which the nucleus could be considered to become reformed on a new site. Plate IX, fig. 3, shows the typical condition met with at this stage. Chromatin which presumably passed out from the karyosome is to be seen lying in the cytoplasm. It will be noticed that the quantity of chromatin lying around the nuclear

membrane is about equal to that shown in a resting nucleus (Plate IX, fig. 2). From this fact it seems that in *Ligniera* only the karyosome passes into the cytoplasm during the akaryote stage. There is no reason for considering that during this stage there is any division of the nucleus. Whether all the chromatin sometimes passes into the cytoplasm could not be definitely observed. It is possible that its staining properties are changed at this stage, and were it possible to differentiate the chromatin part at any rate would be found inside the nuclear membrane.

After the akaryote stage has been passed through the amoeba is ready to form spores. The nuclei of the amoeba divide by two successive divisions which on account of the different size of the spindles are considered to be the reduction divisions. Each of the freshly formed daughter nuclei collects a small quantity of cytoplasm around it. Cell walls are then laid down around these areas and the whole plasmodium becomes converted into a mass of spores. Plate IX, fig. 4, shows an amoeba in the process of forming spores, the nuclei have divided and the cytoplasm is collecting around the daughter nuclei, some of which can be seen in the photograph. The spores themselves are shown in Plate IX, figs. 5 and 6.

Under certain conditions of moisture and temperature the amoeba, instead of forming ordinary spores, becomes cut up into much larger segments, each with a single unreduced nucleus. Around these masses vacuoles are formed and cell walls are laid down later. The nucleus then divides twice as in the case of spore formation. Plate VIII, fig. 4, shows in the two lower cells the large chromatin plate of the first division while in the upper segments are various stages in the second division. This division is a typical mitosis, and though it is not possible to count them, independent chromosomes come upon the equator of the spindle in metaphase. Each of these structures, which may be considered as a zoosporangium, thus contains four, presumably haploid nuclei. Around each of the nuclei a zoospore is organised, which at this stage appear tetrahedral in shape.

It has not been found possible to observe the further fate either of the ordinary spores or of the zoospores nor the method of escape from the host cells in which they are formed. Instances have been found in which some of these spores had escaped from a spore mass and it was noticed that the host cells near by were infected with very young amoebae; it suggests itself that the spores on germination penetrated the wall of the host cell and formed young amoebae in neighbouring cells. The same has been noticed in the case of the zoosporangia. A zoosporangium (Plate VIII, fig. 6) containing a single remaining zoospore was found, but the cells near by were not infected

with young amoebae. It was further noticed that these zoosporangia are found only in the epidermal cells and it seems most likely that the function of these motile spores is to spread the disease to the roots of other plants rather than of propagating it within the cells of the same root.

On one occasion when examining living material a large number of small zoospores were found swimming about in close association with roots containing empty zoosporangia. The size, shape and structure of these zoospores agreed exactly with the spores found in the zoosporangia, especially in the case where all but one of the zoospores had escaped, and the remaining spore had had room to assume its mature shape. (Cf. Plate VIII, figs. 1 and 6.)

These free-swimming zoospores were carefully observed. They are small spherical flagellated organisms about  $4\mu$  in diameter with a single anterior flagellum about the same length as the diameter of the spore. There is a clear area around the anterior end and the nucleus is situated in the centre of the cell. Entrance of the zoospore was observed in one case. The particular root hair entered was swollen at the tip and the cell wall was already slightly broken. There was, however, no previous infection of the fungus either in the root hair or in the epidermal cell to which it was attached. Having entered the root hair through the broken tip the young flagellula swam actively for some considerable distance. For a period of a minute or so it would come to rest and then active motion would begin again. Finally, after reaching very nearly to the base of the root hair motion became spasmodic and finally only the flagellum continued to wave. The flagellum itself soon disappeared and the cell began to send out pseudopodia. The protoplasm became more granular and aggregated about the centre of the cell. The outline of the amoeba gradually became more even and after a short while the typical spindle-shape was assumed. Plate IX, fig. 1, shows two camera lucida drawings made of the living material. The active zoospore is shown outside the root hair and also very soon after entering it.

In the same piece of material several other root hairs were found containing one of these zoospores. These root hairs were not hypertrophied. No hole in the cell wall could be made out through which the zoospore had entered. These zoospores behaved in exactly the same way as described above. The whole process from the entrance of the zoospore to the formation of the spindle-shaped amoeba occupied about half an hour.

It is very probable that the original infection of roots is only by these zoospores and occurs solely through the root hairs, the ordinary spores being a method of tiding the fungus over bad conditions, and for propagation within the cells of the host plant.

## DISCUSSION.

From the evidence obtained from cross-inoculation of healthy plants of a number of Phanerogams with infected roots, it appears certain that the five species of *Ligniera* described by Schwartz are merely host varieties of the same species. It is proposed to call this *Ligniera Junci*, the name given by Schwartz to the first of the series he described.

*Ligniera Junci* is then the only species which is known to occur in this country, and is capable of infecting the roots of a number of aquatic and semi-aquatic flowering plants.

*Ligniera pilorum*, described from France by Fron and Gaillat (6), agrees with *L. Junci* both in its general life cycle, size of spores and zoospore formation. Its cytology has not been worked out, but, as it is found in the roots of *Poa annua* which is a plant frequently attacked by *L. Junci*, there seems little ground for considering it a new species. Fron and Gaillat lay stress upon the hypertrophy produced in the root hairs. If it is a feature of *L. pilorum* that it always causes hypertrophy, then it differs not only from *L. Junci* but also from all the described species of *Ligniera* and indeed cannot rightly be included in the genus which was formed to include only non-hypertrophying forms. If, as seems more likely, the hypertrophy of the root hairs is only occasional, and if the same fungus can be found in roots which have retained their normal size, then it agrees exactly with *L. Junci*, which sometimes attacks swollen root hairs. In such cases root hairs entirely free from the disease are frequently found, and there does not seem to be any direct evidence that this hypertrophy is due to the fungus.

*Ligniera radicalis* resembles *L. Junci* in its life cycle but differs from it in the size of the spores. While those of *L. radicalis* are 4-5  $\mu$  in diameter, those of *L. Junci* are 5-7  $\mu$ . On the other hand, *L. Junci* has been found in the roots of *Callitriche stagnalis*. I have not had the opportunity of examining any slides of *L. radicalis* so that I have no evidence for considering that *L. radicalis* and *L. Junci* are the same species, and in the absence of any definite information upon this point it is better to retain the name *L. Junci* for the British species. From the life history and cytology of the two forms, however, there is strong evidence that they are identical.

*Ligniera verrucosa*, described by Maire and Tison (11, 12) in the roots of *Veronica arvensis*, differs from *L. Junci* most markedly in the warted character of the walls of the spores. Further, instead of being massed together, the spores are more or less free in the host cells.

In their cytology all species of *Ligniera* are very similar.

Schwartz was never able to germinate the spores, nor did he find any stages of zoospore formation in his material. Maire and Tison, however, observed zoospore formation in the roots of *Ligniera radicalis*. Zoospores were also found by Fron and Gaillat. Careful measurements have shown that while the spores themselves are seldom more than  $6\mu$  in diameter, the zoosporangia are often 9 or even  $10\mu$ . Moreover, the wall of the spore is relatively thick while that of the zoosporangium is thin and frequently not spherical.

The life cycle cannot be completely known until the liberation and germination of the spores and zoospores has been followed. Prior to the formation of either spores or zoospores there are two nuclear divisions which appear to be heterotypic and homotypic, the equatorial plate of the first division being much larger than that of the second, as might be expected if this was a reduction division of a spore mother cell. Though these two divisions occur both before spore and zoospore formation, there is a difference in that whereas in zoospore formation the mitotic divisions occur after the segmentation of the amoeba into zoosporangia, in the formation of spores these divisions are completed before the amoeba becomes cut up into uninucleated portions. It is quite impossible to count the chromosomes during the divisions, and therefore there is no direct evidence that this is a reduction division, but by comparison with other types one is forced to such a conclusion. If this is the case the plasmodium at the time of spore formation must be diploid, and the spores and zoospores haploid. It is therefore necessary that at some point after spore formation there is a nuclear fusion. Schwartz suggested that there was a conjugation of the spores on germination. He did not, however, observe the actual process of germination of a spore, and there is still no direct evidence upon this point. In zoospore germination no fusion takes place as far as is known. Careful measurement of the single zoospore remaining in a zoosporangium (Plate VIII, fig. 6) shows that it is exactly the same size as the motile zoospore observed in the act of entering a root hair. This latter zoospore was watched till it assumed the shape of the uninucleated amoeba. It is therefore considered that no conjugation occurred between the liberation of the zoospore and the formation of the uninucleated amoeba within the cell of the new host. Conjugation of amoebae sometimes occurs, but it is common to find a single uninucleated amoeba lying alone in a host cell, and there is no evidence to show that this association of amoebae is in any way a fertilization. From the time the uninucleated amoeba begins to develop in the host cell until the commencement of the reproductive phase the nucleus divides by protomitosis, and



it is most improbable that any change in the number of chromosomes takes place here. The only other place where a nuclear fusion might occur is during the akaryote stage. No workers are at present clear as to what exactly happens during this nuclear change. Osborn considers that the nucleus may only lose the trophochromatin, while the rest of the chromatin still remains within the nucleus, but he realises that to suggest binuclearity in the Plasmodiophorales is unjustifiable without further evidence. It has been suggested that while a certain part of the chromatin passes into the cytoplasm the rest remains within the nuclear membrane, but owing to a difference in the chemical composition its response to stains is altered. Osborn held that there was at any rate an association of the nuclei at this stage and thought that very likely fusion did take place. Schwartz finds no ground for a nuclear fusion at this point in *Sorosphaera Veronicae*, and Maire and Tison, although they offer no suggestion as to where fusion does occur, are certain that it is not during the akaryote stage in *Ligniera*. Moreover, such a conclusion makes any comparison with the Mycetozoa increasingly difficult.

As has already been pointed out, the genus *Ligniera* stands apart from the rest of the Plasmodiophorales in not causing any hypertrophy of the host tissues. In all the other genera such hypertrophy occurs. According to Osborn, *Spongospora subterranea* does not produce any noticeable effect on the potato tuber which was the only organ on which he records the disease. Horne (7) on the other hand states that hypertrophy is produced as a result of the disease. This is further supported by Pethybridge (19), who figures cankerous growths on the roots of potato due to *Spongospora*.

The cytology of *Ligniera* conforms very closely with what is generally found in the Plasmodiophorales. Unfortunately spore germination has not been critically followed in any member of the order. Schwartz tried to germinate the spores of *Plasmodiophora Brassicae* without success. Duggar (3) gives an account of the germination of the spores of *Plasmodiophora* though he does not say whether they are his own observations. This description agrees very closely with what I have observed in *Ligniera Junci*. No one so far has germinated the spores of *Sorosphaera*. Massee (13) states that he had successfully germinated these of *Spongospora* and that the spore gives rise to a single myxamoeba, which is in agreement with my observations on the germination of zoospores in *Ligniera Junci*, and also with what is generally found in the Mycetozoa.

In *Plasmodiophora Brassicae* infection is through the growing point of the root, while both Schwartz (1) and Maire and Tison (9)

consider that in *Sorosphaera Veronicæ* infection occurs through the growing apex of the stem. These workers and also Nawaschin find that the amoebæ of *Sorosphaera* and *Plasmodiophora* have no power of penetrating cell walls. They think that propagation of the fungus throughout the host tissues is simply by division of the host cells. Such cannot be the case in *Ligniera Junci* where infected cells do not appear in any way to be associated together. Moreover, uninucleated amoebæ are frequently found in epidermal cells where no empty spore cases are to be seen along the whole length of the root.

In 1912 Winge<sup>(23)</sup> described *Sorodiscus Callitrichis* in the stem of *Callitriche autumnalis*. The life history very closely agrees with that of *Sorosphaera Veronicæ*. He examined the vegetative nuclei and found that in division the long axis of the chromatin plate was nearly always parallel. This is not the case in *Ligniera Junci*. Maire and Tison<sup>(9)</sup> do not consider that the vegetative soma is a plasmodium, since several times they have observed an amoeba in the metaphase and another in the anaphase of the sporogenous division in the same cell. I have always found that during the vegetative divisions all the nuclei divide simultaneously, which supports Nawaschin's view that a plasmodium is formed. This also agrees with Pavillard<sup>(17)</sup> who states that simultaneous division is constant throughout the endosporous Mycetozoa. Léger<sup>(8)</sup> has described a genus *Sporomyxa* which he related to the Plasmodiophorales living within the coelomic cavity of the imago of *Scaurus tristis*. From his figures it would seem that the nuclei of the amoeba all divide together.

The feature upon which classification is chiefly based is the shape of the spore masses. In *Plasmodiophora* the spores are free, in *Sorosphaera* they are in hollow spheres, while in *Sorodiscus* they are in flattened spheres and ellipsoids. In *Tetramyxa* they are found in tetrads, and in *Spongospora* in spongy masses. In *Molliardia* no spores have so far been found and, as Schwartz points out, when these are found it may be transferred to another genus. In *Sporomyxa* the spores are not arranged in any typical system but the individual spores are ellipsoidal.

It is desirable here to mention two further genera which have been described by Ferdinandsen and Winge, *Clathrosorus*<sup>(5)</sup> and *Ostenfeldiella*<sup>(4)</sup>. *Clathrosorus* was described from some hypertrophied roots of *Campanula rapunculoides*. The material was only roughly fixed and was shrunken by the solution used. The authors claim to have seen both protomitosis and meiosis in their material; however, they give only a brief account of the life history. In *Ostenfeldiella* which was found causing hypertrophy of the stem of *Diplanthera Wrightii* these same authors

were even less fortunate in their material, as it was only preserved in alcohol. Apparently no cytological structure could be made out, and the figures show little more than masses of spores. Both genera, so far as the evidence goes, appear to be closely allied to *Spongospora*. It is much to be regretted that no further description of these genera is forthcoming.

In 1910 Němec<sup>(15)</sup> figured and described a fungus which he called *Sorolpidium Betae* and referred it to the Chytridiales. It was found in the roots of *Beta maritima*. As a result of this paper various attempts have been made to relate the Plasmodiophorales and the Chytridiales. From an examination of Němec's figures it would appear very likely that he was dealing with two independent fungi, the one a *Ligniera*, while the other resembles *Asterocystis* in the structure of the sporangium. Němec does not describe the cytology of his fungus very closely, and does not figure protomitosis. Chytridiaceous fungi are frequently met with in the roots of water plants infected with *Ligniera Junci*. During my examination of roots three species closely related to *Synchytrium* have been found; it is hoped to publish an account of these later. All these Chytridiaceous fungi differ from *Ligniera* in the much coarser and granular structure of the cytoplasm.

Cavers<sup>(2)</sup> was of the opinion that there was no direct relationship between the Chytridiaceae and the Plasmodiophoraceae, but gave them a common ancestry in the *Proteomyxa*.

The members of the Plasmodiophoraceae agree very closely in all important features. Moreover, the characteristic type of protomitosis is not found in any other group of fungi. It is, however, described in some of the Amoebae. Therefore it seems very desirable to keep the Plasmodiophoraceae quite separate from the Chytridiales, and other primitive fungi. If there is any relationship it is most likely through the Mycetozoa.

#### SYSTEMATIC.

Since so much confusion has arisen concerning the systematic position of the genus *Ligniera*, and the species which compose it, it is desirable once again to give the generic and specific diagnoses put forward by Maire and Tison<sup>(12)</sup>, omitting those which can no longer be considered as independent species.

Genus *Ligniera* Maire & Tison.

In cellulis hospitis immutatis parasitans; nec tumores gignens; schizogonia reducta; sporae in acervulos variiforme conjunctae.

*L. verrucosa* Maire & Tison.

Sporis crasse verrucosis, 4-5  $\mu$  diam., in acervulos plenos conjunctis. Habit. in *Veronica arvensi*.

*L. radicalis* Maire & Tison.

Sporis levibus 4-5  $\mu$  diam., in acervulos rarius cavos conjunctis. Habit. in *Callitriche stagnali*.

*L. Junci* (Schw.) Maire & Tison (= *Sorosphaera Junci* Schw., *S. graminis* Schw., *Ligniera graminis* Schw., *L. Bellidis* Schw., *L. Menthae* Schw., *L. Alismatis* Schw., *L. pilorum* Fron & Gaillat).

Sporis levibus 5-7  $\mu$  diam., in acervulos saepe cavos conjunctis. Habit. in radicibus plantarum aquatilium et palustrium.

SUMMARY.

1. Cross-inoculation have been successfully carried out between the five species of *Ligniera* described by Schwartz.

2. The result of these experiments proves conclusively that *Ligniera Junci*, *L. graminis*, *L. Bellidis*, *L. Menthae* and *L. Alismatis* are only host varieties of the same species, for which it is proposed to retain the name *Ligniera Junci* on grounds of priority.

3. The life history of *Ligniera Junci* has been followed, and agrees in all essential points with that of the other two species *L. radicalis* and *L. verrucosa* described by Maire and Tison.

4. Reasons are given for considering that *Ligniera pilorum* is identical with *L. Junci*.

5. Zoospore formation has been observed in *L. Junci*. The process of infection by the zoospores in the root hairs has been followed and their movements have been followed from the time of entrance in the tip of the root hair to the formation of a spindle-shaped amoeba.

6. Reasons are given for considering that zoospores are the only method provided for the infection of fresh host plants; the spores being a resting stage for tiding the fungus over unsuitable weather conditions and for propagating it within the cells of the infected plant.

7. Reduction division has been observed at the time of formation of both spores and zoospores. Conjugation has never been found.

8. The stages in protomitosis have been followed, and the process so far as is yet known agrees with that described by Nawaschin in *Plasmodiophora*. Further work on this subject is in progress.

9. The genus *Ligniera* is a member of the Plasmodiophoraceae,

and has many points of relationship with the Mycetozoa. It differs from any other member of the group in not causing any hypertrophy of the host tissues.

10. The Latin diagnoses of the genus, and the recognised species as given by Maire and Tison are appended.

The work was done in the Botanical Department of King's College, London. I wish to take this opportunity of thanking Professor Gates for much helpful advice and criticism. I also desire to thank Dr E. J. Schwartz for his valuable assistance and advice throughout the investigation.

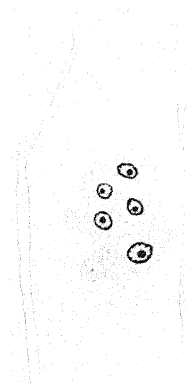
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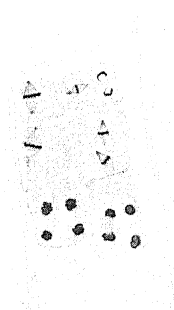
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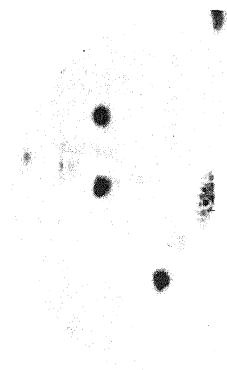
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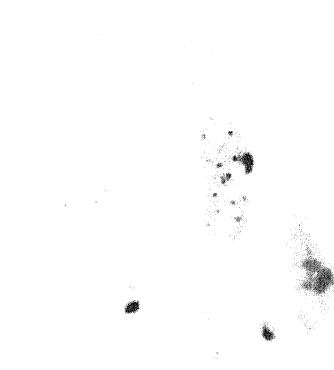
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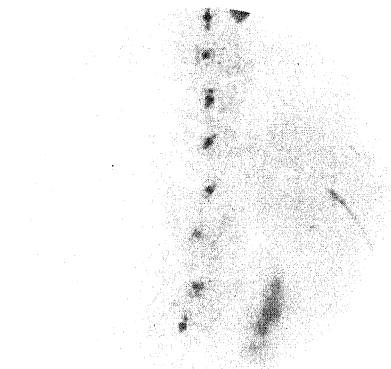




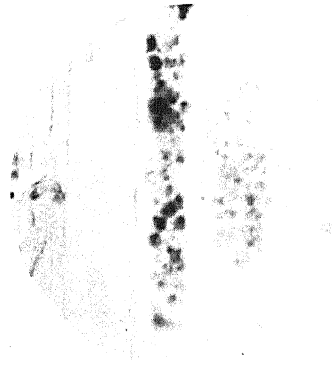
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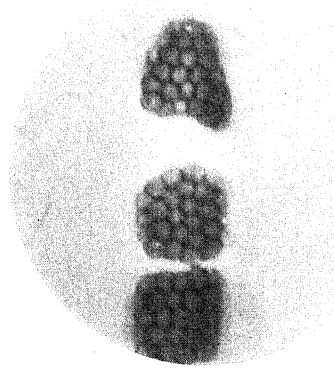
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#### DESCRIPTION OF PLATES.

The drawings were made with a camera lucida at table level, with a tube length of 160 mm. In the drawings a Koritska 2 mm. apochromatic objective (n.a. 1.30) was used with Zeiss compensating ocular 10 (figs. 1-3 and 5), with ocular 20 (fig. 4) and with ocular 7 (fig. 6). The photomicrographs were made with a Zeiss Seidentopf Photographic Eyepiece at tube length 115 mm. using a Zeiss 4.2 mm. (n.a. 0.65) (figs. 1, 2, 4 and 5), Zeiss 2 mm. (n.a. 1.4) (fig. 3) and Koritska 2 mm. (n.a. 1.3), (fig. 6).

#### PLATE VIII.

- Fig. 1. Two stages in the entrance of the zoospore in the root hair. Both drawings were made on living material at short intervals from one another.  $\times 650$ .  
Fig. 2. A resting amoeba with five nuclei. Shows the chromatin around the nuclear membrane and the large conspicuous karyosome.  $\times 650$ .  
Fig. 3. The akaryote stage, showing the chromatin extruded into the cytoplasm, no karyosome, but the chromatin still remaining around the nuclear membrane.  $\times 650$ .  
Fig. 4. Two stages in the reduction division prior to the formation of zoospores. The plasmodium has already broken up into segments and then the nuclei have divided; the two on the left show the metaphase of the first division while those on the right are stages in the second division.  $\times 1350$ .  
Fig. 5. Zoosporangia showing the four resting nuclei of the zoospores.  $\times 650$ .  
Fig. 6. Empty zoosporangia, showing one with four zoospores still remaining and another with a single zoospore. Compare this zoospore with Fig. 1.  $\times 480$ .

#### PLATE IX.

- Fig. 1. A young uninucleated amoeba lying in a root cell, showing the large conspicuous karyosome.  $\times 350$ .  
Fig. 2. A resting plasmodium.  $\times 350$ .  
Fig. 3. Protomitosis in a plasmodium showing the typical "cruciform division." Owing to the difference of level a photograph of this stage can only show one or two nuclei in focus at one time.  $\times 1750$ .  
Fig. 4. Segmentation of the plasmodium into spores. The reduction divisions have taken place and the cytoplasm is being reconstituted around the nuclei.  $\times 350$ .  
Fig. 5. A mass of spores.  $\times 350$ .  
Fig. 6. Another mass of spores showing the structure of the wall.  $\times 800$ .

### NOTES ON A PYCNIDIAL FUNGUS ASSOCIATED WITH A DYING- BACK OF APPLE BRANCHES.

(With 7 Text-figs.)

By E. A. Southey and F. T. Brooks.

#### FIELD OBSERVATIONS.

A FEW years ago some Lord Derby apple trees at Meldreth, Cambridgeshire, were brought to our notice, large and small branches of which were gradually dying back. In the early stages of disease the bark was affected in long strips on one side

of the branch, with sometimes a sharp line of demarcation between the healthy and diseased tissues so that something in the nature of a canker was produced (cf. Fig. 1).

Occasionally, death of the bark in a long branch appeared to begin at the place of attachment of a spur which had been killed, but, more frequently, it could not be ascertained where

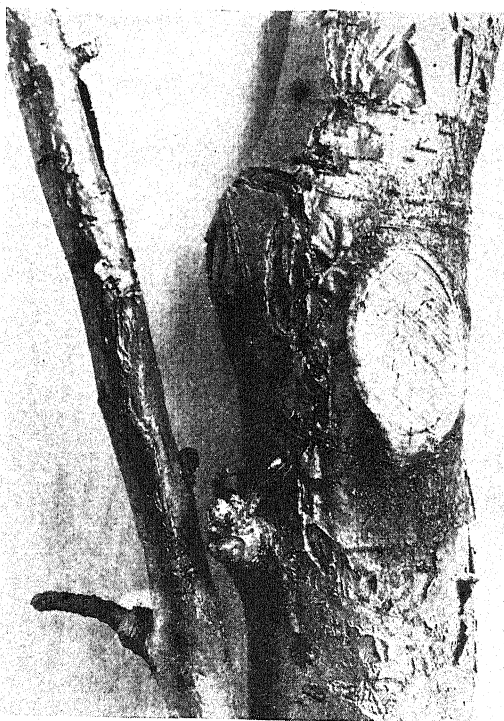


Fig. 1. Photograph of diseased bark on a Lord Derby apple tree.  
Natural size.

the disease had commenced. The disease frequently spread round the whole of the bark, and branches so affected died back. A characteristic feature of the dead bark was the manner in which the superficial tissues peeled away from the underlying parts.

A few trees of other varieties of apples such as Lane's Prince Albert and Cox's Orange Pippin have been seen to be affected by the same disease in other parts of the country, but the disease is uncommon.

THE FUNGUS ASSOCIATED WITH THE DISEASE.

On dead parts of branches from which the superficial tissues had peeled away fructifications of a fungus were invariably found. These were small, more or less spherical bodies, greyish

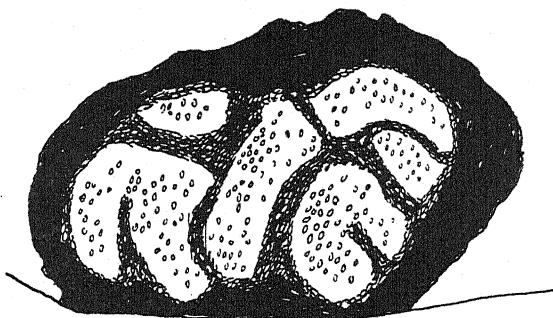


Fig. 2. Long. section through a naturally occurring stroma.  $\times 100$ .

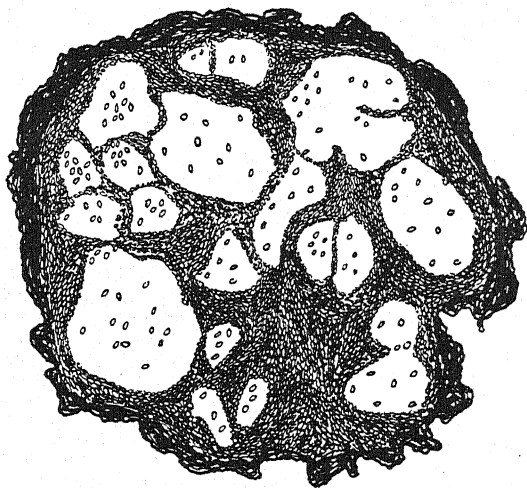


Fig. 3. Trans. section through a naturally occurring stroma.  $\times 100$ .

black in colour, and about 1 mm. in diameter. These fructifications are stromata in which are embedded one or several pycnidia, more than one chamber being occasionally connected with a single ostiole (cf. Figs. 2 and 3).

The spores are formed from the extremities of short, tapering conidiophores, which line the base of the pycnidium. The spores

are oval or sub-globose in shape with rounded ends, have a large, conspicuous guttule, and are mostly hyaline, although a few (less than 1 per cent.) become light brown with age (cf. Fig. 4).

The spores vary in size from  $9\text{--}14 \times 7\text{--}10\mu$ , averaging  $10 \times 7.5\mu$ . The spores exude from the ostioles as creamy masses.

Although the fungus is confined to the bark at first, it ultimately penetrates the wood, causing it to become discoloured.

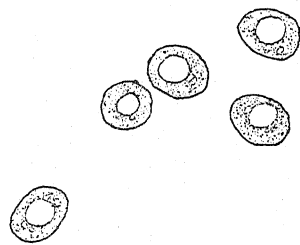


Fig. 4. Spores from a naturally occurring stroma.  $\times 800$ .

#### PURE CULTURES OF THE FUNGUS.

Cultures of the fungus were established from single spores upon potato agar, apple wood-extract agar, and apple twigs. The fungus grew well and produced fructifications on all these media. On sterilised apple twigs, however, the stromata bearing the pycnidia were often considerably elongated and projected from the surface as horn-like processes. Upon examining the pycnidia formed in culture it was seen that although some contained only the type of spore found in nature, others contained also large numbers of hyaline, straight, rod-like spores,  $3\text{--}4 \times 1\mu$  in size. These rod-like spores were never encountered in naturally-occurring pycnidia. As the cultures were continued, some pycnidia produced only the rod-like type of spore. The large pycnosporos formed in culture frequently contained several guttules instead of the one characteristic of those found in nature.

Cultures of the fungus obtained from the junction of diseased and healthy tissues behaved in the same manner as those arising from single spores.

#### GERMINATION OF THE SPORES.

Spore germination was studied in hanging drop cultures of apple wood-extract and in thin films of apple wood-extract.

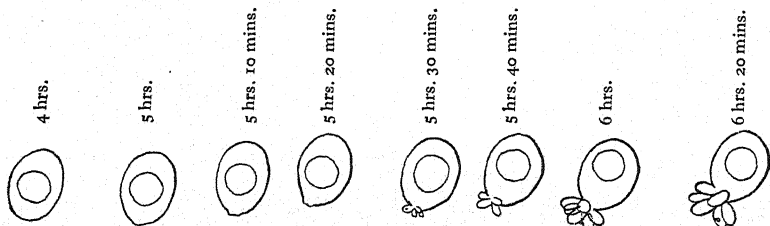


Fig. 5. Stages in the usual method of spore germination, the intervals after the time of placing the spores in the culture medium being indicated.  $\times 1400$ .

agar spread on glass slides. The mode of germination was of two principal kinds, the commonest being of a peculiar nature.

After a few hours in the culture medium the spore swells and begins to show a slight prominence at one end. Shortly afterwards, bud-like protuberances arise in association with this prominence; these ultimately form secondary spores which break away from the mother spore. They are hyaline and measure  $4.5-8.5 \times 2-5.5 \mu$  (cf. Fig. 5).

After another twenty hours these "budded," secondary spores

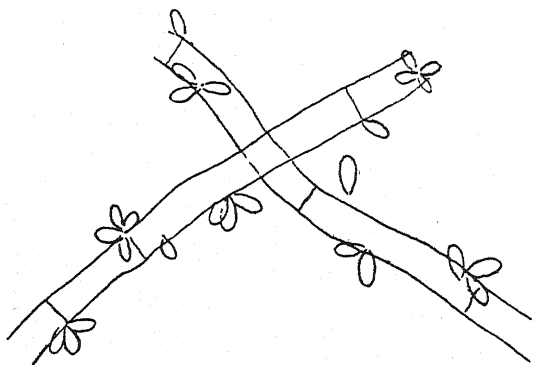


Fig. 6. Spores borne directly on the mycelium formed after germination.  $\times 1400$ .

germinate from one or both ends to form hyphae. Meanwhile from the opposite end of the mother spore a germ tube usually arises, which develops into a mycelium. The mycelium, whether derived direct from a pycnospore or a "budded" spore, in turn forms other spores laterally, arranged singly or in groups, similar in size to the "budded" spores (cf. Fig. 6).

In the other type of germination, which is exceptional, two or three germ tubes of the usual kind arise from the pycnospores (cf. Fig. 7).

There are modifications in detail between these two types of germination.

We did not succeed in germinating the small, rod-like spores

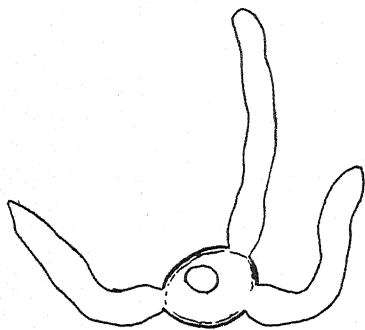


Fig. 7. Another, but exceptional, type of spore germination (after 24 hours).  $\times 1400$ .

## SYSTEMATIC POSITION OF THE FUNGUS.

There is considerable uncertainty about the systematic position of this pycnidial fungus. It probably belongs to the Sphaerioidaceae-Hyalosporae section of the Sphaeropsidales, although a few of the spores are brownish in colour. The choice of genus would appear to lie between *Dothiopsis*, *Cytospora*, and *Dothiorella*. The naturally-occurring fructifications contain either one or several pycnidia, so that in this respect the distinction between *Dothiopsis* and *Cytospora* breaks down. The distinction between *Cytospora* and *Dothiorella* is based upon the size and shape of the spores, a not very satisfactory criterion.

As far as we are aware the small rod-shaped spores produced in some pycnidia under cultural conditions have not been described as occurring in either of the genera mentioned above, but as they do not occur under natural conditions, little stress need be laid on them. These small spores are similar in many respects to the "B" spores of many species of *Phomopsis*.

We are inclined to refer the fungus to the genus *Cytospora*, especially as Mr J. Ramsbottom has kindly called our attention to a species *Cytospora fructorum*, occurring on fruits of *Pyrus* in Belgium, which has been described by É. and Ém. Marchal in the *Bulletin de la Société Royale de Botanique de Belgique*, LIV (1921), 125, and which agrees closely with the fungus now under consideration. Our fungus diverges slightly from that described by É. and É. Marchal in that the spores are not exactly globose and are slightly larger. It seems best, however, provisionally to assign the fungus to this species. An effort was made to obtain material of *Cytospora fructorum* from Monsieur Marchal, but unfortunately it was not available.

## INOCULATION EXPERIMENTS.

A large number of inoculation experiments with pure cultures of the fungus were carried out in wounds made in branches of Lord Derby apple trees, but in no case did infection result. It was invariably found that the fungus, after penetrating the tissues slightly, soon ceased to develop further, and the wound was ultimately occluded. The pathogenicity of this fungus is therefore still in doubt. Observations in the field indicated that the fungus was behaving as a slow-growing parasite, and it may be that it can only act parasitically in trees which have been weakened by some other cause. Recent work in the pathology of fruit trees indicates that there are several fungi which act destructively only when the trees are in a debilitated condition. The Lord Derby apple trees on which the fungus

chiefly occurred were growing amongst rank grass in a rather poorly drained, heavy soil overlying chalk. Such conditions may have led to considerable weakening of the trees, allowing the fungus to attack them.

#### SUMMARY.

1. A die-back of the branches of Lord Derby apple trees in Cambridgeshire is described.

2. A pycnidial fungus is constantly associated with this disease. There is some uncertainty about the identity of the fungus, but it is provisionally named *Cytosporella fructorum*, a species which Él. and Ém. Marchal have described as occurring on fruits of *Pyrus* in Belgium.

3. The methods of germination of the pycnosporos are described. The commonest mode of germination is peculiar in that secondary spores are budded off from one end of the pycnosporos.

4. Under cultural conditions many pycnidia give rise to small rod-like spores which have not been found in nature.

5. Inoculation experiments with the fungus were unsuccessful in reproducing the disease. It is likely that the fungus behaves as a parasite only when the trees are already weakened from some other cause.

## THE INHERITANCE OF SPORE SIZE IN *COPRINUS STERQUILINUS*.

(With 2 Text-figs.)

By W. F. Hanna, M.Sc. (Alberta).

### I. INTRODUCTION.

THE possibilities of increasing the food value and productivity of the higher plants by selection of superior individuals must have been recognised in a general way by all primitive agricultural peoples. However, the fundamental principles which are involved in the process of selection have only recently been studied. Johannsen's\* classical researches on pure lines form one of the foundation stones upon which the present-day conception of selection has been built. He chose for his experiments a certain variety of the common garden bean, *Phaseolus vulgaris nana*, known as the Princess bean. In 1901, he planted a number of bean seeds of different sizes and known weights. At the end of the season each plant was harvested separately and an exact record was kept of the weight of each seed. When the weights

\* Johannsen, W. Elemente der exakten Erblichkeitslehre. Jena, 1909.



of the mother beans were compared with the weights of the progeny, it was evident that the original population from which the mother beans had been selected was a mixed one and that selection had resulted in the sorting out of a number of already existing types or "pure lines." Johannsen then proceeded to determine the effect of the selection of plus and minus variants within his pure lines. In these experiments he employed nineteen pure lines, and the work was carried on for six years, from 1902 to 1907 inclusive. Each year, every pure line was represented by two lots of plants, one a plus strain grown from large beans and the other a minus strain grown from small beans. Continued selection, however, failed to produce permanent departure in either direction and the offspring of the plus and minus variants exhibited complete regression to the mean of the particular line. A series of similar experiments using the character length and breadth of seed gave the same result. Johannsen, therefore, concluded that individual variations within a pure line are not inherited and that selection within such a line is without effect.

Johannsen's experiments and those of other investigators have shown that in the higher plants the species must be regarded as consisting of various groups of individuals which possess a general similarity but which, nevertheless, differ from one another in respect to minor heritable characters. Environmental conditions may bring about further variation in the individuals making up each group or pure line; but, on the basis of Johannsen's work, modifications of this kind will not be transmitted to succeeding generations and will no longer appear when the influences which brought them into existence have ceased to operate. Heritable modifications within pure lines, therefore, can arise only by mutation.

Biologic forms are known to be of relatively common occurrence in many species of parasitic fungi. Furthermore, Arthur\*, Gäumann†, Levine‡, and others have shown that biologic specialisation is accompanied in many instances by a certain degree of morphological differentiation. By using single-spore cultures Brierley§ demonstrated the existence of a number of races, morphologically distinct, within species of *Botrytis*, *Penicillium*, and *Stysanus*. On the basis of such evidence, we may conclude that species of fungi are not homogeneous in com-

\* Arthur, J. C. Cultures of Uredineae in 1916 and 1917. *Mycologia*, ix (1917), 295-312.

† Gäumann, E. Ueber die Formen der *Peronospora parasitica* (Pers.) Fries. *Beih. z. Bot. Centr. (Abth. 1)*, xxxv (1917-18), 395-533.

‡ Levine, M. N. A statistical study of the comparative morphology of biologic forms of *Puccinia graminis*. *Jour. Agr. Res.* xxiv (1923), 539-567.

§ Brierley, W. B. Experimental studies in the specific value of morphological characters in the fungi. *Proc. Lin. Soc. London*, Oct. 1918.

position but, like those of the higher plants, are made up of a number of separate races or pure lines.

Very little experimental evidence is available as to the value of continued selection within pure lines of fungi. On *a priori* grounds it might be expected that selection in fungi would obey the same general laws as in the higher plants, *i.e.* that continued selection within a pure line would fail to bring about any permanent departure in either morphological or physiological characters. It is well known that fungi possess a high degree of variability and that, under cultural conditions, many species are extremely unstable. The possibilities of selection in fungi, therefore, would seem to be worthy of serious consideration. Burkholder\* has recently shown that long-continued culturing of *Fusarium Martii Phaseoli* on artificial media may bring about changes in both morphological and physiological characters, which persist for some time after the fungus has been again transferred to its host plant. The strain with which he worked was isolated from a diseased bean root, and the first culture was made from a single spore. Six years of culture on artificial media was effective in bringing about distinct changes in the colour of the mycelium and in the size and number of the conidia produced and, in addition, the fungus had lost much of its former virulence. When this attenuated form was allowed to infect the bean plant and was then re-isolated, its original characters were partly restored and, after two such passages, the organism was found to possess all its former virulence. Rosenbaum†, on the contrary, found that selection of large conidia of *Phytophthora infestans* for five generations was ineffective in producing any change either in the size of the conidia or in the relative numbers of large and small conidia.

La Rue has carried out extensive experiments on the effect of selection in *Pestalozzia Guepini*‡ and *Helminthosporium teres*§. All the cultures used by him originated from single spores. Selections according to progeny in *Pestalozzia Guepini* were made for length of spore for ten generations and for length of spore appendages for twenty-five generations; but, at the end of the selection period, no permanent modification had been

\* Burkholder, W. H. Variation in a member of the genus *Fusarium* grown in culture for a period of five years. Amer. Jour. Bot. XII (1925), 245-253.

† Rosenbaum, J. Studies of the genus *Phytophthora*. Jour. Agr. Res. VIII (1917), 233-276.

‡ La Rue, C. D. The results of selection within pure lines of *Pestalozzia Guepini* Desm. Genetics, VII (1922), 142-201.

§ La Rue, C. D. The results of selection within pure lines of the genus *Helminthosporium*. Paper read before Amer. Assoc. for the Advancement of Science, Dec. 1925.

brought about in respect to either of these characters. Similarly, when individual large and small spores were selected, in one experiment for six generations and in another experiment for ten generations, no increase or decrease in the average size of the spores was effected. The experiments with *Helminthosporium teres* were carried out for thirty generations, and the selections were made on the basis of spore length. A long line was propagated from the longest individual spore of each generation, and a short line was propagated from the shortest individual spore of each generation, while a third unselected control line was propagated from spores taken at random. The mean spore length of each generation was obtained by measuring 150 spores from each culture. Throughout the experiment the average length of the spores in the three lines paralleled one another in a remarkable manner, and the selecting of long and short spores seemed to be without effect in altering the average spore length of the species.

From the experimental evidence that is available it would appear that the spore characters of fungi are comparatively stable and cannot be readily altered by selection. Final conclusions, however, will be warranted only after a careful study of many groups of fungi.

The size, shape, and colour of the reproductive bodies of the higher fungi are generally regarded as reliable characters for systematic purposes. In the classification of the Agaricaceae much importance is placed upon macroscopic characters, such as the shape and colour of the pileus and the attachment of the gills; but spore size is also known to be of great value in the identification of species, and in most modern works on mycology spore measurements are included in the description of each species. It is well known that there may be considerable variation in the size of the spores collected from a single fruit body. Differences have also been noted in the average size of spores from different fruit bodies of the same species. Buller\* determined the average diameters of the spores of three wild fruit bodies of *Amanitopsis vaginata* by measuring 50 spores from each fruit body and found them to be  $10.19 \mu$ ,  $10.87 \mu$ , and  $11.65 \mu$  respectively. When systematic works are consulted for the spore dimensions of a particular species, it is frequently found that there is considerable disagreement between the measurements recorded by different authors. Differences of this kind might reasonably be expected if the spore sizes given were based on the measurement of a few spores from a single fruit body. There is also the possibility that within species of the Agaricaceae there may exist numerous strains or pure lines

\* Buller, A. H. R. Researches on Fungi, 1 (1909), 161.

each having a characteristic size of spores. As far as the writer is aware, however, no experiments on the inheritance of spore size in this group of fungi have been made. The present paper is a contribution to this subject.

The fungus selected for experiment was *Coprinus sterquilinus*, a large coprophilous species which occurs commonly on horse dung in both Europe and North America. It has been described and fully illustrated by Buller\*. Miss Mounce† has shown that this species is homothallic, a fact since confirmed by Brunswik‡.

In the experiments to be recorded later, *Coprinus sterquilinus* was successfully cultivated in monosporous culture for several successive generations. In one series of experiments each generation was started from as small a spore as possible, and in another series of experiments each generation was started from as large a spore as possible. Both series of experiments were carried out with a view to determine whether or not the ordinary fluctuating variations in spore size are inherited.

## II. METHODS.

In studying the effect of selection on a particular organism, careful consideration must be given to the genetic purity of the material from which selections are to be made. If a normally self-fertilised species such as the bean is employed, the progeny of a single seed may be regarded as a pure line. If, however, a cross-fertilised species is employed, selection of self-fertilised individuals for several generations will be necessary before an approximately homozygous condition is reached.

In view of recent progress in our knowledge of sex in the higher fungi, *Coprinus sterquilinus* may be regarded as a particularly suitable species for use in an experimental study of the inheritance of spore size. The work of Miss Mounce§, supplemented by the nuclear studies of Brunswik|| has shown that a single spore of *Coprinus sterquilinus*, when germinated on dung agar, gives rise to a haploid mycelium characterised by simple septa and isolated nuclei and that, after a few days, this haploid mycelium spontaneously becomes diploid, the diplophase being indicated by the presence of clamp connections

\* Buller, A. H. R. *Researches on Fungi*, III (1924), 177-257.

† Mounce, Irene. Homothallism and the production of fruit bodies by monosporous mycelia in the genus *Coprinus*. *Trans. Brit. Mycolog. Soc.* VII (1921), 198-217.

‡ Brunswik, H. *Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomycetengattung Coprinus*. K. Goebel's Botanische Abhandlungen, V (1924), 1-152, Jena.

§ Mounce, Irene. *Loc. cit.* pp. 203-205.

|| Brunswik, H. *Loc. cit.* pp. 15-19.

and the occurrence of the nuclei in pairs. The nuclei in this diploid mycelium divide conjugately. After a few weeks the diploid mycelium gives rise to perfect fruit bodies. In each young basidium there are two nuclei of opposite sex which fuse together. The fusion nucleus divides twice, as shown by Buller\*, and the four nuclei pass upwards through the sterigmata into the four spores. Thus the life history of the fungus passes through all its possible stages beginning with a single spore; in other words, *C. sterquilinus* is homothallic. The progeny of a single spore of *C. sterquilinus*, therefore, may be regarded as a pure line.

Cultures of *C. sterquilinus* were made from spore deposits collected from a number of wild fruit bodies which appeared on dishes of horse dung kept in the laboratory. A spore deposit was obtained as follows. When a fruit body had begun to shed its spores, the pileus was removed by cutting through the stipe at the level of the pileus periphery. The pileus was then pinned through its centre to a small cork which had previously been attached with sealing wax to a circular glass plate. A sterilised glass slide was placed in the bottom of a crystallising dish and the glass plate, with the pileus suspended on its lower side, was placed as a cover over the dish. In this way the pileus came to hang at a height of from two to three inches immediately above the glass slide. As soon as the slide had become coated by a thin spore deposit—of such a nature that, when viewed under the microscope, the spores were seen to be fairly close together although not touching one another—it was removed, labelled, and placed in a cardboard case.

Single spores were removed from the dry spore deposit on a glass slide by the dry-needle method† and were sown singly in hanging drops of nutrient gelatine having the following composition:

Dextrose	10 gm.	Sodium chloride	5 gm
Peptone	10	Gelatine	100
Beef extract	5	Water	1000 cc.

Out of a total of 433 spores selected from 50 different fruit bodies and placed in hanging drops of gelatine for germination, 101 or 23·3 per cent. germinated. There was considerable variation in the viability of spores from different fruit bodies. In some fruit bodies the number of spores germinating was higher than 23·3 per cent., while in other fruit bodies the percentage was much lower than this and the spores could be induced to

\* Buller, A. H. R. *Researches on Fungi*, III (1924), 208.

† Hanna, W. F. The dry-needle method of making monosporous cultures of Hymenomycetes and other fungi. *Ann. Bot.* xxxviii (1924), 791–794.

germinate only with the greatest difficulty. In general, the small spores were less viable than the large ones. Of the 433 spores selected and sown 229 were between  $10.7\ \mu$  and  $16.7\ \mu$  in length, and of these only 16.2 per cent. germinated; the remaining 204 spores were between  $17.2\ \mu$  and  $23.3\ \mu$  in length, and of these 31.4 per cent. germinated. The larger spores, therefore, germinated about twice as well as the smaller ones.

Miss Mounce\* has already referred to a paper by Miss Baden† on the germination of spores of *C. sterquilinus*. Miss Baden found that the spores of *C. sterquilinus* germinated only when certain bacteria about  $1.2\ \mu$  in length and  $0.8\ \mu$  in breadth were present in the culture medium. Other longer bacteria inhibited the growth of the mycelium. She states that "hanging-drop cultures were made with and without the bacteria. In those with the bacteria germination took place within twenty-four hours, but it never occurred at all without them. These experiments seem to show that the bacteria are in some way necessary for the germination of the spores." She further found that the spores of *C. sterquilinus* did not mature until about three weeks after they had been shed but that if dried for two days at  $40^{\circ}\text{C}$ ., they would germinate at once. This she regarded as an adaptation on the part of the spores to retard germination until the substratum had become fairly dry and such fungi as *Mucor* had disappeared.

The results which Miss Baden obtained have never received any confirmation. In 1911, A. H. R. Buller and S. G. Churchward carried out a series of experiments‡ which proved conclusively that the spores of *C. sterquilinus* will germinate satisfactorily in a number of sterile synthetic media. Later, Miss Mounce§ germinated the spores and obtained fruit bodies without difficulty under sterile conditions of culture. In the present study, many strains of *C. sterquilinus* have been grown in pure culture throughout several generations. The spores were found to germinate at room temperature in hanging drops of sterile nutrient gelatine or dung agar. Moreover, no difference was observed in the germination of spores which had just been shed and those which had been kept for several weeks. On one occasion, ten spores which had just been shed were placed in hanging drops of sterile nutrient gelatine and within twenty-four hours all had germinated. My own observations, therefore, confirm those of Buller and Churchward and of Miss Mounce; they seem

\* Mounce, Irene. Loc. cit. p. 213.

† Baden, M. L. Observations on the germination of spores of *Coprinus sterquilinus* Fr. Ann. Bot. xxix (1915), 135-142.

‡ Communicated to me in ms. by Professor Buller.

§ Mounce, Irene. Loc. cit. pp. 203-205.

to provide convincing evidence that Miss Baden was entirely mistaken in concluding (1) that the spores of *C. sterquilinus* germinate only in the presence of certain bacteria, and (2) that they are not ready to germinate when they are shed.

Fruit bodies were obtained by transferring the monosporous mycelia to dishes of sterile horse dung. Small crystallising dishes, five inches in diameter and three inches in depth, were found to be suitable for the purpose; they were half-filled with horse dung, covered with Petri-dish covers, and autoclaved for one hour at fifteen pounds pressure. A small piece of sterile dung agar was placed on the dung in each crystallising dish and a hanging drop of gelatine with its monosporous mycelium was transferred to the dung agar. By this method fruit bodies were generally obtained twenty-five days from the time of spore germination, although cultures were frequently observed to fruit in twenty-two days. No culture fruited in less than twenty-two days.

All spore measurements were made with the Poynting Plate Micrometer. This instrument has been described and illustrated by Buller\* who was the first to employ it in biological research. The instrument used for the experiments recorded in the present paper was attached to a Watson microscope equipped with a mechanical stage which could be rotated when necessary. The mechanism for rotation was not present in the original instrument used by Buller. The Poynting Plate Micrometer combines speed of manipulation with a high degree of accuracy and has given most satisfactory results throughout the present work.

The method finally adopted for measuring spores was somewhat different from that which is generally employed and, therefore, will be described in detail. In preliminary experiments the spores were mounted in a drop of water on a glass slide, covered with a coverglass, and measured wet; this is the method usually adopted. It was noticed, however, that the true lengths of some spores were difficult to obtain owing to the fact that, in water, the long axes of the spores were not parallel to the face of the glass slide. If the cover glass was pressed down slightly with a view to remedying this defect, the spores often became appreciably compressed, thus bringing about a change in the ratio of length to breadth. It was then found that *the spores could be measured much more accurately when dry than when wet*. As is recorded elsewhere, the author working in conjunction with Buller† discovered that spores of *C. sterquilinus* which have fallen from a pileus and have settled upon a dry glass slide always have their long axes parallel to the surface

\* Buller, A. H. R. Researches on Fungi, 1 (1909), 158-163.

† Ibid. III (1924), 224-230.

of the slide, and that each spore is held in this position by a thin colourless adhesive layer on its more rounded side which is always directed towards the surface of the glass. These dry spores never alter in shape or position. When viewed under the microscope, they appear perfectly regular in outline and are perfectly still, so that they can be measured with great accuracy. All spore dimensions which are recorded in the following experiments are based on the measurement of dry spores collected on glass slides.

The dimensions which are given for the dry spores may be compared with similar measurements presented by other workers for spores mounted in water by referring to the following table:

Table I.

Condition of spores	Mean length of spores in microns	Mean breadth of spores in microns
Dry	17.4	12.6
Wet	18.6	11.8

The sizes given in Table I were calculated from the measurements of 36 spores. Each spore was measured separately on the dry slide and was then transferred with the needle to a drop of water and measured again. On the basis of these measurements, a calculation shows that dry spores, on being wetted, increase in length 6.9 per cent., and decrease in breadth 6.4 per cent.; but that the product of their length and breadth remains practically constant.

In comparing the sizes of the spores from different fruit bodies, lengths only have been considered. The breadth of the spores, however, may be calculated from the data in Table I by taking the ratio of length to breadth of dry spores as 1 : 0.72 and that of wet spores as 1 : 0.63.

The number of measurements which must be made to determine accurately the average length of the spores from a given fruit body depends upon the variability in size of the spores which are to be measured. If the range of variability is small, a few measurements may suffice; while, if the variability is great, a correspondingly larger number of measurements will have to be made. For two fruit bodies, one with an average spore length of  $18.7\mu$ , and the other of  $18.4\mu$ , the probable error in the measurement of 100 spores was found to be  $\pm 0.08\mu$ . When groups of 20 spores were measured from the same two fruit bodies, the experimental error in determining the average spore length was found to be as high as  $\pm 0.5\mu$ . In the following experiments the average lengths recorded for spores of different fruit bodies are based on the measurement of 100 spores



from each fruit body. When this number of spores is employed, the error in determining the average length should be small even for fruit bodies with a wide range of variability in spore size.

### III. VARIATION IN THE SIZE OF SPORES OF DIFFERENT WILD FRUIT BODIES.

An examination of spore deposits of *Coprinus sterquilinus* collected from wild fruit bodies, which appeared upon horse dung cultures in the laboratory, showed that a wide variation in size may be found among the spores from a single fruit body. Considerable differences were also observed in the mean size of the spores produced by individual fruit bodies. The extent of these variations is brought out in Table II which gives the mean spore size and the limits in variation in spore size for five wild fruit bodies.

Table II.

Fruit body	Mean spore length in microns	Range of variation in spore length in microns
1	19.9	17.7-21.9
2	18.7	16.3-21.4
3	18.7	13.5-21.4
4	16.1	13.0-19.5
5	15.9	11.6-18.1

Table III.

Authority	Length in microns	Breadth in microns
Baden† ...	15-18*	8-12
Carleton Rea‡ ...	14-23	9-14
Kauffman§ ...	18-25	—
Murrill   ...	18	12
Ricken¶ ...	18-22	12-14

\* The length actually given is 0.15 mm.-0.18 mm., but this is obviously intended for 0.015 mm.-0.018 mm.

† Baden, M. L. Loc. cit. p. 140.

‡ Rea, Carleton. British Basidiomycetae, p. 501. Cambridge, 1922.

§ Kauffman, C. H. The Agaricaceae of Michigan, Lansing, 1 (1918), 211.

|| Murrill, W. A. Mycologia, III (1911), 167.

¶ Ricken, A. Die Blätterpilze, 1, 57. Leipzig, 1915.

As may be seen from an inspection of Table II, the limits of variation in the lengths of individual spores from different fruit bodies are from 11.6  $\mu$  to 21.9  $\mu$ , while the mean lengths of the spores from different fruit bodies range from 15.9 to 19.9  $\mu$ . Converted into lengths of wet spores with the help of the data for dry and wet spores given in connection with Table I, the lengths just referred to become 12.4  $\mu$  to 23.4  $\mu$ , and 17.0  $\mu$  to 21.3  $\mu$  respectively. It is, therefore, quite evident that the measurement of any number of spores from a single fruit body

would be entirely insufficient to give a true picture of either the range in variation or the mean size of the spores for the species as a whole.

Reference to a number of works showed that mycologists are by no means agreed as to the size of the spores of *C. sterquilinus*. The size of the spores, as given by several authorities, is set down in Table III.

The range of spore length given is from  $14\ \mu$  (Carleton Rea) to  $25\ \mu$  (Kauffman). Whether the limits of variation given by these authorities apply to the spores of a single fruit body or to those of several fruit bodies is uncertain and, in the absence of such information, these data are of limited value.

#### IV. EXPERIMENTS ON THE INHERITANCE OF SPORE SIZE.

To determine whether or not individual variations in spore size are inherited in succeeding generations, single spore selections were made from the wild fruit bodies Nos. 1 to 5 of Table II and from another fruit body not included in this table. The results of these experiments are shown in Table IV. From fruit body No. 1, large spores were selected for five generations; from fruit body No. 2, large spores were selected in three lines of experiment and small spores in two lines for five generations; from fruit body No. 3, small spores were selected for three generations; from fruit bodies Nos. 4 and 5, small spores were selected for two generations; from fruit body No. 6, small spores were selected for five generations. The original spore deposit of fruit body No. 6 was unfortunately lost before measurements from it could be made.

As may be inferred from a study of Table IV, no particular relationship appears to exist between the size of the spore selected and the mean size of the spores from the resulting fruit body. Furthermore, although the original fruit bodies from which selections were made varied considerably in respect to the size of their spores, these differences do not appear to have been inherited as they have not been consistently retained in the succeeding generations.

The results presented in Table IV may be studied more easily by comparing the mean size of the spores from all the fruit bodies produced by monosporous mycelia arising from large spores with similar data for all the fruit bodies produced by monosporous mycelia arising from small spores. This information is summarised in Tables V and VI.

The mean length of the spores from the 21 fruit bodies resulting from large-spore selections was  $18.1\ \mu$ , while an equal number of fruit bodies resulting from small-spore selections had a mean spore length of only  $17.7\ \mu$ . At first sight these data seem to

Table IV. *The effect of selecting large and small spores of Coprinus sterquilinus throughout several successive generations.*

( ) indicates length of mother spore in microns. ○ indicates mean length of 100 spores from the progeny fruit body.

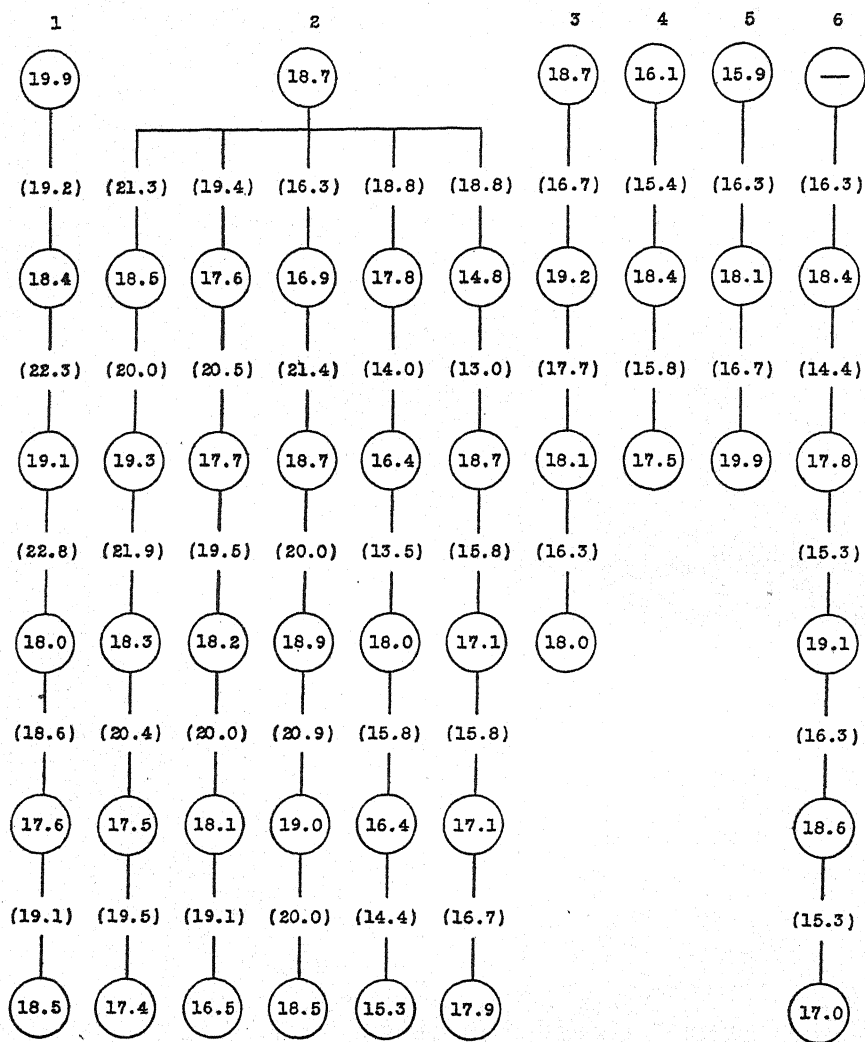


Table V. *Fruit bodies produced by monosporous mycelia arising from large spores.*

Generation	Number of fruit bodies	Mean spore length of progeny fruit bodies in microns
1	5	17.4
2	4	18.7
3	4	18.4
4	4	18.1
5	4	17.7

Table VI. *Fruit bodies produced by monosporous mycelia arising from small spores.*

Generation	Number of fruit bodies	Mean spore length of progeny fruit bodies in microns
1	5	18.2
2	6	18.1
3	4	18.1
4	3	17.4
5	3	16.7

suggest that the selection of small spores has had a slight effect in reducing the size of the spores in the progeny. However, when considered in relation to the differences in the mean size of the spores from individual fruit bodies, the effect of selection in modifying spore size appears to be without significance.

The coefficient of correlation between the size of the mother spores and the mean size of the spores produced by the progeny fruit bodies was calculated by Karl Pearson's formula. The value obtained was  $0.257 \pm 0.097$ . When considered in relation to the probable error, the evidence of correlation is very slight. The following conclusions may therefore be drawn: (1) the original wild fruit bodies from which selections were made did not represent a number of strains possessing spores of a particular size, and the differences in spore size which they exhibited were merely the result of fortuitous variations which were not inherited in succeeding generations; and (2) the continued selection of large and small spores for five generations did not result in any material increase or decrease in the size of the spores produced by the progeny fruit bodies.

In view of the experiments which have just been described, spore size may be regarded as a stable character for systematic purposes provided sufficient consideration is given to variability in the size of spores from individual fruit bodies.

The large number of spore measurements which were made in studying the inheritance of spore size in *C. sterquilinus* provide very suitable material for an analysis of the variations in spore size in this species, and an account of these results will now be given.

### V. VARIATION IN SPORE SIZE DURING THE SPORE-DISCHARGE PERIOD.

Considerable variation may be found in the mean size of the spores collected from a fruit body at different times during the spore-discharge period. Measurements of the mean length of spores collected from three fruit bodies at the beginning, middle, and end of the spore-discharge period are given in Table VII.

Table VII.

Period of spore discharge	Fruit body <i>A</i> Mean length of spores in microns	Fruit body <i>B</i> Mean length of spores in microns	Fruit body <i>C</i> Mean length of spores in microns
Beginning ...	17.4	17.3	18.7
Middle ...	18.4	17.7	18.7
End ...	20.0	16.9	19.1

From the data embodied in Table VII it is clear that there is considerable variation in the mean length of the spores produced by a fruit body at different times during the spore-discharge period. While in fruit bodies *A* and *C* there is an increase in the size of the spores produced at the end of the spore-discharge period, in fruit body *B* there is a decrease. To what these differences are due is by no means evident. That spores which develop under conditions of desiccation may be subnormal in size has been shown by Cotton\* who found that there was a gradual diminution in the size of the spores shed by a pileus of *Stropharia semiglobata* which had been severed from its stipe and placed in the warm dry air of a room: the spores collected during the first hour of spore discharge measured 18  $\mu$  in length, while those collected in the eighty-third hour measured only 12  $\mu$  in length and were pale in colour. In the present investigation the spore deposits of *Coprinus sterquilinus* were collected from pilei suspended in closed crystallising dishes, so that very little drying of the gills, if any, could have taken place. Moreover, in *Coprinus sterquilinus*, as shown by Buller†, the spores on the gills all attain their maximum size before the pileus expands and begins to shed its spores from below upwards. It is clear, therefore, that transpiration during the spore-discharge period cannot have affected the size of the spores liberated. The spores in the Coprini ripen on each gill in succession from below upwards and are shed from below upwards; so that the spores collected from a fruit body at the beginning, middle, and end of the spore-discharge period are derived respectively from the

\* Cotton, A. D. On the production of imperfectly developed spores in the Agaricaceae. Trans. Brit. Mycolog. Soc. iv (1914), 298-300.

† Buller, A. H. R. Researches on Fungi, III (1924), fig. 73, p. 184.

lower parts, middle parts, and upper parts of the gills. Possibly, therefore, the variations in spore size given in Table VII were due to an irregular flow of food materials to the hymenium during its development.

#### VI. A CORRELATION BETWEEN THE SIZE OF THE SPORES AND THE WIDTH OF THE PILEUS.

The size of the spores produced by a fruit body is correlated to some extent with the size of the pileus of the fruit body. Buller\* found that the spores of dwarf fruit bodies of *Coprinus lagopus*, while of about the same breadth as those of larger fruit bodies, are distinctly shorter. He has also shown† that the hairy scale cells on the pilei of fruit bodies of *Coprinus lagopus* are larger in large fruit bodies than in small fruit bodies. Table VIII shows the relation between the diameter of the pileus and the size of the spores produced for twenty-three fruit bodies of *C. sterquilinus*.

Table VIII.

Number of fruit bodies considered	Diameter of pileus in cm.	Mean length of spores in microns
2	1	15.5
2	5	16.8
5	6	18.3
4	7	18.4
9	8	18.2
1	9	18.3

From a consideration of the data set forth in Table VIII we may conclude: (1) that fruit bodies with small pilei produce small spores; (2) that the size of the spores increases with the diameter of the pileus until the latter reaches 7-8 cm. which is normal for the species; and (3) that a further increase in the diameter of the pileus is not correlated with any further increase in the size of the spores.

#### VII. VIABILITY OF LARGE AND SMALL SPORES.

It is evident from the data which have already been presented that spores of *C. sterquilinus* vary greatly in size. In the many spore deposits examined, the largest spore found measured  $23.3\mu$  in length, while the smallest spore found measured  $10.7\mu$  in length. The largest and smallest spores observed to germinate were respectively  $22.8\mu$  and  $12.6\mu$  in length. Extremely large or extremely small spores must be regarded, therefore, not as abnormal or imperfectly formed

\* Buller, A. H. R. Researches on Fungi, II (1922), 86.

† Ibid. III (1924), fig. 141, p. 320.

structures, but as viable reproductive bodies capable of performing their normal functions.

As already pointed out in Section II, larger spores were found to germinate about twice as well as smaller ones. Hence we must conclude that small spores are less viable than large ones.

#### VIII. THE GENERAL RANGE OF VARIATION IN SPORE SIZE FOR THE SPECIES.

The extent of the variation in size of the spores of *C. sterquilinus* is shown by the frequency curve in Fig. 1. This curve was constructed from measurements of the lengths of a hundred

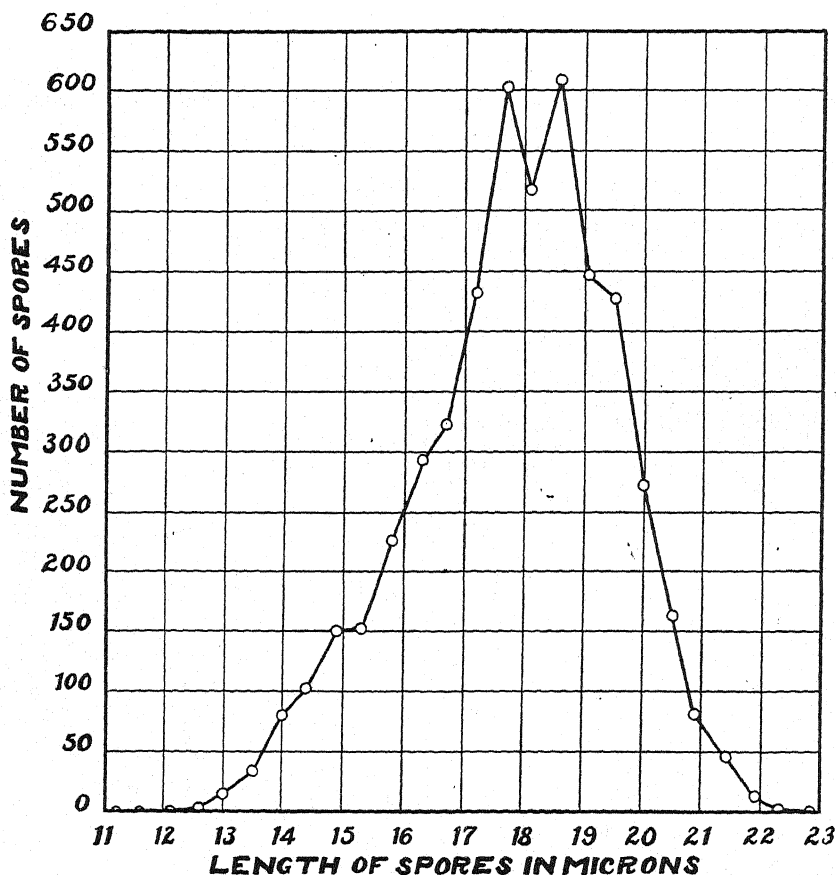


Fig. 1. Frequency distribution of spores from fifty fruit bodies of *Coprinus sterquilinus*, according to spore length. One hundred spores from each fruit body were measured. The class interval is  $0.465 \mu$ .

spores from each of fifty fruit bodies. As is shown graphically, the 5000 spores measured vary in length from  $11.2\ \mu$  to  $22.8\ \mu$  with a mean at  $17.8\ \mu$ . Notwithstanding the large number of spores measured, the distribution is very irregular. This unevenness, undoubtedly, is due to the fact that the curve is multimodal in nature, with the spores from each fruit body, or from each group of similar fruit bodies, occupying a particular position on the curve.

Fig. 1 presents a general picture of the complete range of variation in spore size for the species, but gives little information as to the frequency distribution of the spores from the different types of fruit bodies which go to make up the compound curve. In view of the difficulty of showing frequency curves for a range of fruit bodies having modes for spore length from  $14.4\ \mu$  to  $20.5\ \mu$ , only three types of fruit bodies will be considered: (1) those having modes for spore length of  $14.4\ \mu$ – $15.3\ \mu$ ; (2) those having modes for spore length of  $15.8\ \mu$ – $16.7\ \mu$ ; and (3) those having modes for spore length of  $20.0\ \mu$ – $20.5\ \mu$ . The first group is made up of four fruit bodies, the second of three fruit bodies, and the third of three fruit bodies; and 100 spores from each fruit body were measured. The three types of frequency distribution are shown in Fig. 2. Two of the curves are asymmetrical in form; the curve for spores from large-spored fruit bodies has a negative skew, while that for spores from small-spored fruit bodies has a positive skew. The distribution of the spores from fruit bodies having spores with modes for length from  $15.8\ \mu$ – $16.7\ \mu$  approaches in form the normal frequency curve. A measure of the skewness may be obtained from the formula  $\frac{\text{mean mode}}{\sigma}$ , where  $\sigma$  refers to the standard deviation.

The coefficients of skewness, as calculated from this formula, are as follows:

$$\begin{aligned}\text{Group 1 (Modes } 14.4\ \mu\text{--}15.3\ \mu) &= +0.294 \\ \text{,, 2 (Modes } 15.8\ \mu\text{--}16.7\ \mu) &= 0 \\ \text{,, 3 (Modes } 20.0\ \mu\text{--}20.5\ \mu) &= -0.557\end{aligned}$$

In fruit bodies having modes for spore length of  $14.4\ \mu$ – $15.3\ \mu$ , the lower limit of spore size seems to have been reached. If smaller spores than these were to form on the gills of a fruit body of *C. sterquilinus*, they would probably not mature and, therefore, would not be shed by the fruit body. For this reason, a frequency curve for spores from such fruit bodies falls off rapidly on the lower side and gradually on the upper side, as is shown by the coefficient of skewness. In fruit bodies having modes for spore length of  $15.8\ \mu$ – $16.7\ \mu$ , a wide range of variation is possible both below and above the mode, so that spores from



such fruit bodies show a normal frequency distribution. In fruit bodies having modes for spore length of  $20.0\ \mu$ – $20.5\ \mu$ , the upper limit of spore size is approached and variation is possible only in the direction of smaller spores; a frequency distribution

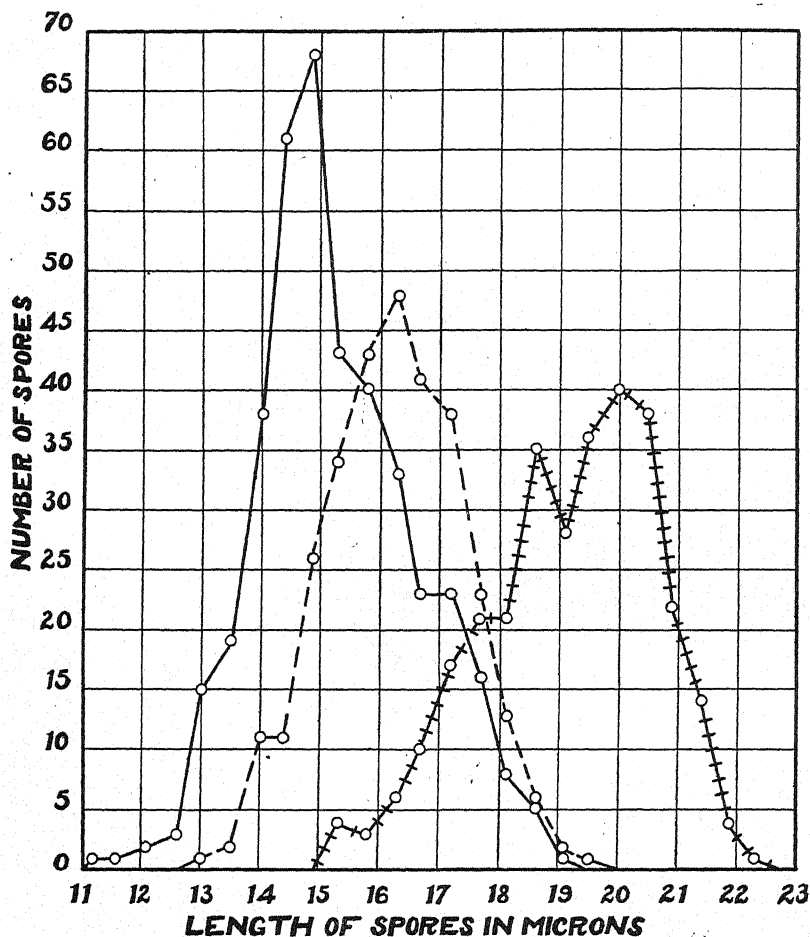


Fig. 2. Frequency distribution of spores from three groups of fruit bodies of *Coprinus sterquilinus*, according to spore length. Group 1, four fruit bodies with modes for spore length  $14.4\ \mu$ – $15.3\ \mu$ ; group 2, three fruit bodies with modes for spore length  $15.8\ \mu$ – $16.7\ \mu$ ; group 3, three fruit bodies with modes for spore length  $20.0\ \mu$ – $20.5\ \mu$ . One hundred spores from each fruit body were measured. The class interval is  $0.465\ \mu$ .

for spores from such fruit bodies, therefore, shows negative skewness. If the fruit bodies which have been studied had

represented distinct races of *C. sterquilinus* differing from one another in respect to the size of their spores and breeding true for such a character, these distinct types of frequency distribution would not have been expected to occur. The nature of the variations in spore size in the fruit bodies studied, therefore, would seem to provide additional evidence that fruit bodies of *C. sterquilinus* which produce large spores do not differ genetically from those which produce small spores. Variations in spore size must be attributed rather to the particular physiological conditions under which the fruit bodies develop.

#### IX. SUMMARY.

1. An experimental study has been made of the inheritance of spore size in *Coprinus sterquilinus*, one of the homothallic Hymenomycetes.

The character on which selection was based was length of spore. The original spore selections were made from six wild fruit bodies. In one series of experiments, each generation was propagated from the smallest spore which could be found; in another series of experiments, each generation was propagated from the largest spore which could be found. Selections from three fruit bodies were carried on for five generations, those from one fruit body for three generations, and those from two fruit bodies for two generations. Records were kept of the length of each motherspore, and of the mean length of the spores from the progeny fruit body of each monosporous mycelium. All spores were measured when dry with the Poynting Plate Micrometer.

2. The attempt to produce large-spored strains and small-spored strains in pure lines of *Coprinus sterquilinus* by the continuous selection of large and small spores respectively failed. No satisfactory evidence of the inheritance of individual variations in spore size was found. In this respect the spores of *Coprinus sterquilinus* resemble the seeds of the garden bean *Phaseolus vulgaris nana* investigated by Johannsen, the conidia of *Phytophthora infestans* investigated by Rosenbaum, and the spores of *Pestalozzia Guepini* and *Helminthosporium teres* investigated by La Rue.

3. There is considerable variation in the mean length of the spores produced by a fruit body at different times during the spore-discharge period.

4. Fruit bodies having small pilei produce smaller spores than those having pilei of the normal size or larger.

5. Since the mean size of the spores of different fruit bodies of one and the same species of Hymenomycete vary considerably, the spore size given by systematists for determining hymenomycetous species ought to be based on measurements of the spores of a number of fruit bodies obtained in different places.

6. The largest and smallest spores found were respectively  $23.3\mu$  and  $10.7\mu$  in length; the largest and smallest spores observed to germinate were respectively  $22.8\mu$  and  $12.6\mu$  in length.

7. The percentage germination of spores between  $17.2\mu$  and  $23.3\mu$  in length was 31.4; that of spores between  $10.7\mu$  and  $16.7\mu$  in length was only 16.2. Thus larger spores were found to germinate twice as well as smaller ones.

8. Frequency curves of spore length are presented for fruit bodies having modes for spore length of (1)  $14.4\mu$ – $15.3\mu$ , (2)  $15.8\mu$ – $16.7\mu$ , and (3)  $20.0\mu$ – $20.5\mu$ . The relation between the forms of these curves and the nature of the variation in spore size in fruit bodies of *Coprinus sterquilinus* is discussed.

9. Miss Baden's conclusions (1) that spores of *Coprinus sterquilinus* germinate only in the presence of certain bacteria and (2) that they are not ready to germinate when they are shed have not been confirmed.

The foregoing investigation was carried out in the Botanical Laboratory of the University of Manitoba during the tenure of a scholarship awarded by the Canadian Society of Technical Agriculturists; a grant in aid of this work was also made by the Canadian Honorary Advisory Council for Scientific and Industrial Research. The problem was suggested by Professor A. H. R. Buller, whose valuable advice and stimulating criticism is gratefully acknowledged.

## RHACOPHYLLUS B. & BR.

(With Plate X and 2 Text-figs.)

By T. Petch, B.A., B.Sc.

In the *Fungi of Ceylon*, Berkeley and Broome instituted a new genus of Agaricaceae, *Rhacophyllum*, giving the generic description, "Pileus tenuissimus, tenerrimus; lamellae in fragmenta oblongo-obtusa flexuosa divisae." The single species, *Rhacophyllum lilacinus*, was described as follows:

"301 *R. lilacinus* B. & Br. (No. 825, cum icone.)

"On dead wood, twigs, etc. Peradeniya, Dec. 1868, Jan. 1869.

"Pileus cylindrical or digitaliform, lilac, striate, or even[,] split more or less at the margin; stem dilated at the base, attenuated upwards; gills replaced by numberless oblong, irregular, waved, obtuse lobes of the same colour as the pileus.

"It is possible that there may be two species; but the number of specimens gathered at present is very small. *Pterophyllum*,

Mont., agrees somewhat in character; but it is closely allied to *Panus*, while this is more closely to *Coprinus*."

Berkeley and Broome's description was drawn up chiefly from the paintings sent by Thwaites. The figures were reproduced in *Journ. Linn. Soc.* xiv (1873), plate 2. Figure s which Berkeley and Broome described as "gills, with processes more highly magnified" is marked by Thwaites on the original, "Shred from the pileus with laminae," which, on examination of the specimens, is perceived to be a very different statement from that of Berkeley and Broome.

On Thwaites's second sheet of figures, he marked one, "probably something else." This is a group of three contiguous pilei, sessile on the substratum, the stalks not having yet expanded. The colour is brownish white, different from that of the mature specimens, and it was probably for this reason that Thwaites suggested that they might be a different species. This figure is reproduced in *Journ. Linn. Soc.* (*loc. cit.*), as figure e (left), but one would hardly realise from the reproduction that the original shows three distinct cylindric pilei, acute at the apex. Berkeley and Broome's suggestion that there were two species in the type was evidently a repetition of Thwaites's note. These immature specimens are not in Herb. Peradeniya. It would seem probable from the figure that they were merely immature examples of *Rhacophyllus lilacinus*.

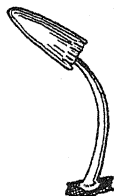


Fig. 1. *Rhacophyllus lilacinus*, nat. size.

*Rhacophyllus* has been regarded as an agaric damaged by insects or as an abnormality. F. Ludwig, in 1908, in a paper on abnormal fructifications in Basidiomycetae, suggested that *Rhacophyllus* was a polyporoid *Coprinus*, i.e. a *Coprinus* in which the lamellae were replaced by pores, but it is evident that he had not seen a specimen.

In 1901 Patouillard described similar specimens from Tunis. These grew on rotten tree trunks, arising in groups from a white mycelium. The fully-developed fungus had a campanulate, greyish fawn pileus, 8-10 mm. high, striate and deeply lacinate at the margin, and a fragile, white, cylindric, fistulose stalk, up to 2 cm. high and 1.5 mm. diameter. The gills were rose purple, some reaching the apex of the stalk, and others shorter.

The trama of the pileus was reduced to a very thin membrane. The gills consisted of series of small fleshy particles, flattened, approximately of the same size, united by their edges, adhering only feebly to one another and readily separating. The substratum was usually powdered with these fragments of the gills.

These particles were irregularly orbicular, from 150 to 300  $\mu$

diameter, 70–100  $\mu$  thick, thinning out towards the edge and somewhat biconvex. They consisted entirely of angular cells,  $10 \times 6 \mu$ , hyaline, thin-walled, with refringent contents. The contour of these cellular masses was well defined. A few irregular filaments were observed, binding them to one another, or they were united by their edges in groups of three or four.

In the earlier stages, the fructification (receptacle) was entirely enclosed in a universal veil which soon disappeared. The margin of the pileus was straight and in contact with the stalk.

Transverse sections showed that in young specimens the gills were continuous, but in slightly older specimens the formation of the cellular masses had already begun and they appeared as compact, white islets, arranged in rows, united by delicate hyphae which gradually disappeared as the fungus developed.

Patouillard regarded these structures as bulbils. Instead of gills, the fungus produces sheets of biconvex bulbils, loosely united by their edges.

The hyphae of the bulbils were at first cylindric, short, and continuous. Subsequently they became torulose, transversely septate; and finally were divided into angular cells which formed a compact pseudoparenchyma.

Patouillard stated that this production of bulbils occurred at all points where normally basidia would be formed, and thus involved the interlamellar spaces as well as the gills. The entire thickness of the gills was transformed.

No normal agarics were obtained in company with the Tunisian examples. Similar specimens were received by Patouillard from Guadeloupe, differing only in that the pileus was more conical than in the Tunisian examples, and that they grew in tufts instead of being scattered. The specimens from Guadeloupe, however, were accompanied by a single example, which the collector (Duss) had noted as being the normal state of the fungus. The latter was a *Psathyra*, near *Psathyra gyroflexa*. Consequently Patouillard considered that the forms in which the gills were converted into bulbils were probably derived from a *Psathyra* or *Psathyrella*, taking into account their general shape and aspect.

In 1913, Patouillard described similar specimens from Tonkin, and assigned them to *Rhacophyllus*. The Tonkin specimens were very small, only 2 mm. high, globose before expansion with a regularly cylindrical stalk which arose from a small disc. The bulbils were pyriform, attached to the interior surface of the cap by the broader end, with the free acute tip directed towards the centre; they were 0.25 to 0.3 mm. long, and were arranged side by side in a single row. The specimens from Tunis and Guadeloupe differed from those from Tonkin in having the

bulbils smaller and more rounded, and arranged in five or six rows.

Patouillard was of opinion that these bulbiferous forms were derived from different agarics, in all probability related to *Psathyrella*.

Berkeley and Broome's reference to *Pterophyllus* was considered by Patouillard, who states that *Pterophyllus Bovei* is a true *Pleurotus*, and that the masses on the faces of the gills are superficial accumulations of spores.

The bulbils of Patouillard's specimens (preserved in alcohol) were studied by Moreau, who states that the majority of the cells contain two nuclei, the normal number in the cells of the pileus of a Basidiomycete. The two nuclei approach one another and fuse, so that the cells become uninucleate. The nucleus then undergoes mitosis, presenting a spindle with two chromosomes and two centrosomes, a typical mitosis of a Basidiomycete. In consequence of this mitosis, the primitive number of nuclei in the cells is restored. Each nucleus then divides again, so that the cells become quadrinucleate.

So far, the nuclear history of the cell comprises a karyokinesis, followed by a chromosome reduction and two successive mitoses. This is exactly the same as occurs in basidia. Each cell of a bulbil, from the point of view of its nuclear history, is homologous with a basidium.

Two of the four nuclei degenerate, and thus the primitive number of nuclei is again restored. Sometimes a supplementary mitosis occurs and six nuclei are found in a cell.

The foregoing details concerning the bulbils are all taken from Moreau's account.

Moreau also states that a perforation sometimes appears in the wall between two adjacent cells, and the protoplasm of the two cells unites.

#### CULTURES.

In July, 1916, specimens of *Rhacophyllus lilacinus* were found at Henaratgoda, growing on a recently felled log of *Ficus nervosa*. Parts of the log were cut off and conveyed to Peradeniya, where they were kept in a sink in the laboratory and periodically watered. Some further specimens developed in transit, and a succession of others appeared until the middle of September. The specimens were scattered or clustered, and there was no superficial mycelium. No normal agarics appeared on the wood, which was in two pieces, weighing together half a hundred-weight. As the fructifications matured, the bulbils fell and formed small patches round the base of the stalk.

Bulbils were sown on blocks of *Ficus* wood in Roux tubes on

September 4th. The blocks were cut from a newly felled branch, boiled (weighted down), fixed in the tubes with a plug of cotton wool at one side, and then sterilised three times. Good growth occurred at once, a white mycelium spreading over the wood and extending along the sides of the tube. The initial stages of the sporophores were observed on September 14th, and the sporophores were fully developed in one tube on September 16th. Four tubes out of six contained fully-developed sporophores on September 17th, while in the other two immature sporophores were present. The mycelium was somewhat feathery, with radiating strands. Up to fifty sporophores in various stages of development were present in a single tube, while over a dozen were expanded at the same time, filling the bore of the tube. Sporophores developed on the cotton wool and on the strands on the sides of the tube. They did not exceed one centimetre in height.

In one Roux tube from which the cotton wool plug had been omitted, the superficial mycelium was scanty, but sporophores were nevertheless developing on September 17th.

Bulbils were also sown on similar blocks resting on wet cotton wool plugs in boiling tubes, after sterilisation as before. In this series, the wood was more saturated with water than in the foregoing. Of six tubes started on September 12th, growth of mycelium was evident on September 17th only in two tubes which were drier than the others. On September 19th, growth was evident in three. On September 21st, the first two were producing fructifications; two others showed mycelium only; while nothing was evident in the two wettest. On September 26th, fructifications were present in four tubes, but no growth had occurred in the two wettest tubes. The experiments were then brought to a conclusion by the unexpected transfer of the writer to administrative duties.

In no case did any normal agarics occur in these cultures, nor did any appear on the original *Ficus* blocks.

*Rhacophyllus lilacinus* would appear to prefer a porous, well-aerated wood as a substratum. The wood of *Ficus nervosa*, and of the *Ficus* used in the above experiments, is very porous. It will be noted that the only failures were in tubes in which the wood was wetter than in the others.

Bulbils placed in hanging drops of water rapidly produced a mycelium. The hyphae arose chiefly from the marginal cells of the bulbil, and radiated outwards over the surface of the cover glass. Lateral branches were produced, those from adjacent radial hyphae fusing with one another and forming cross branches, so that the whole mycelium had the appearance of a cobweb.

Thus *Rhacophyllus lilacinus* can be reproduced from the bulbils, and all the examples obtained in that way have been identical in structure with the parent fungus. The enforced termination of the experiments prevented trials on other media.

#### DESCRIPTION OF THE FUNGUS.

The fungus is usually cylindric at first, rounded or pointed at the apex, sometimes ovoid, or sometimes in small examples globose. It is enclosed in a very thin universal veil, which disappears, or is represented by a few shreds on the pileus, when the fungus expands in nature, but may partly persist as a small shallow cup at the base of the stalk in examples grown in culture. The unexpanded fungus has the appearance of an immature *Psathyrella* or *Coprinus*, and its expansion consists chiefly of a lengthening of the stalk.

The expanded fungus is up to 3 cm. high, but the majority of specimens do not exceed half that height. The pileus is up to 1 cm. high, conical, or conico-cylindric, or conico-campanulate, with a rounded apex, purple-rose, feebly striate, lacerate at the margin. The stalk is white, up to 3 cm. high, smooth, expanded at the base, attenuated upwards, up to 1 mm. diameter in the middle, arising from a flat tomentose disc. The fructifications may be either scattered or clustered. In nature, in the specimens hitherto seen, they do not arise from an evident mycelium, but in culture they may spring directly from the wood or from strands of mycelium which spread over the wood and the sides of the tube.

In the positions usually occupied by the gills of an agaric, the fungus bears sheets of more or less flat "bulbils," arranged edge to edge in the same plane. The plane which may be considered to represent one gill may contain over thirty of these bulbils, varying enormously in size and shape. In general, they are irregularly angular with rounded corners, sometimes long triangular, sometimes lobed, sometimes evidently composed of two or more fused together by their narrow edges. They are up to  $40\ \mu$  thick, and vary in size from  $0.3 \times 0.2$  to  $1 \times 0.4$  mm. In the unexpanded fungus, the plane of a single gill shows some bulbils extending from the tissue of the cap to the stalk, and others in a row of two or three across the gill. Some are united to the cap, others to the stalk, while others are free from either. The bulbils which occupy the position of a single gill are not, as a rule, in contact with one another at their edges, but are separated by spaces,  $4\text{--}16\ \mu$  broad, which contain a web of hyphae. The bulbils are not quite flat, but, as noted by Berkeley and Broome, somewhat undulating. They are not biconvex. They are pale purple-rose when mature, and to them the



apparent colour of the pileus is due. When dry they are pale purple-brown.

The pileus expands to an angle of about  $20^\circ$  on each side of the vertical. The bulbils then fall out, leaving a thin, hyaline, membranous cap. The tissue of the cap is about  $0.1$  mm. thick at the apex, diminishing to  $20\text{--}25\mu$  at the lower edge, and is composed of radial, parallel, septate hyphae, with segments varying from  $4$  to  $8\mu$  in diameter.

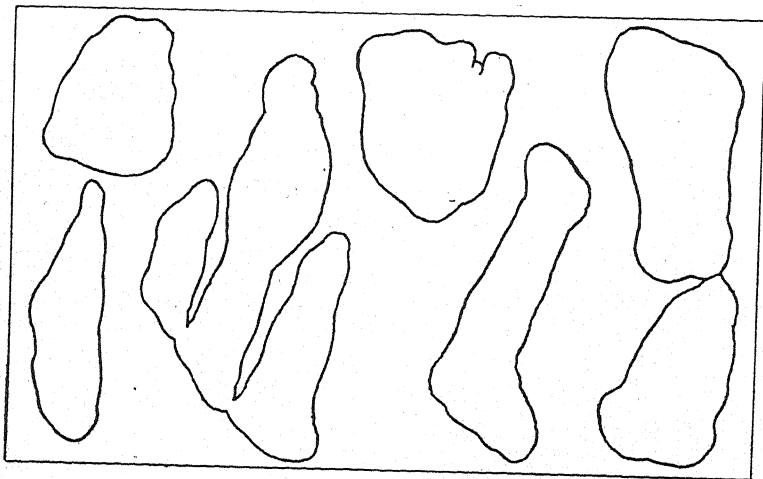


Fig. 2. Bulbils of *Rhacophyllum*,  $\times 150$ .

The bulbils are of almost uniform thickness, with rounded edges, and are composed of thick walled, polygonal cells. At the narrow edge, there may be a layer of parallel hyphae, two to four hyphae thick, but in general only the disorganised remains of these hyphae persist, with sometimes a short, free fragment of a hypha extending from the bulbil. This disorganised layer does not cover the flat sides of the bulbil, and there is no differentiated cortex. Under pressure, most of the cells of the bulbil readily separate. The individual cells are more or less hexagonal,  $6\text{--}9\mu$  diameter, or angularly ovoid, up to  $12 \times 8\mu$ , with a wall about  $2\mu$  thick. Some remain united in short chains of two to five, or in plates of six or eight. Some cells bear a thin solid projection up to  $6\mu$  long from one angle; others may have two, from adjacent angles. These projections lie between the walls of the adjacent cells. It is not clear what they represent.

Although the bulbils are composed of thick walled cells, they can scarcely be styled sclerotia. The cells are arranged more or

less in rows from one side of the bulbil to the other. The bulbil is not formed from irregularly intertwined hyphae, as are sclerotia in general. Nevertheless, they are sclerotoid in nature, though not in mode of formation.

A longitudinal section, almost median, of an example just prior to expansion is given on Plate X, fig. 1. Owing to the curvature of the stalk, the latter and the bulbils are cut obliquely. As the bulbils do not lie exactly in radial planes, and as they are usually curved, it is difficult to obtain a section showing a complete radial series, but the left-hand side of the figure shows one approximately. The annular appearance of the bulbil near the apex is due to the fact that it was concave and the central portion has been cut off. Similarly, the sickle-shaped bulbil a short distance below the former is only the lower part of one. At the lower right-hand corner, the planes of the radial series are twisted, and the bulbils are cut transversely. This example was embedded in celloidin, and the hyphae which run between the bulbils are not visible.

Cross-sections of the developing fructification show that it is solid (Plate X, fig. 2), the plate-like bulbils being embedded in a plectenchyma which fills all the spaces between them. There are no interlamellar cavities. The plectenchyma descends to the outer edge of the pileus, the clefts in the margin of the latter being continued inwards for a short distance. When the stalk lengthens and the pileus expands, the hyphae of the plectenchyma are torn apart and the bulbils fall out. Some of the hyphae persist, forming loose strands extending from the cap (Plate X, fig. 7).

Of the rows of bulbils seen in a cross-section, some extend from the pileus to the stalk, others are in contact with one or the other, while others are not in contact with either. It is, however, obvious, that as the bulbils do not form a continuous tissue in a vertical gill plane, the number which appear to be attached to the pileus or the stalk will vary at different heights, especially as the edge of a bulbil is frequently curved. But it is also clear that there are broad and narrow planes of bulbils, corresponding with the long and short gills of an agaric. In one instance, of fifty-five radial lines of bulbils in the cross-section, twenty-six were attached to the stalk, and forty-eight to the pileus. Sometimes the bulbil appears to be merely in contact with the stalk or the pileus. In other cases it is attached by a narrow zone of parenchyma, the cells of which are smaller than those of the pileus or stalk respectively.

To make this account clearer, we may provisionally accept the view that the rows of bulbils are homologous with the lamellae of an agaric. For convenience, the bulbils which lie in the same

radial vertical plane will be referred to as a lamellar series, or as a lamella.

The youngest example examined was 0.8 mm. high, and 0.5 mm. diameter. Its height was probably abnormal for the stage of development attained, owing to the fact that it arose partly beneath the base of a larger specimen. In this, the stalk primordium was well developed, and composed of rather large longitudinal hyphae. The outline of the pileus was evident as a zone, convex above, but the pileus was not separated from the universal veil. In a tangential section, a short distance from the median plane, the tissue above the stalk primordium was 100  $\mu$  thick. Of this, the thickness of the universal veil was 50  $\mu$ , the latter being distinguished from the pileus primordium by its larger irregular cells. From the pileus primordium, a series of vertical, rather distant trabeculae extended downwards into the fundamental tissue; these, like the pileus primordium, stained more deeply with haematoxylin than the surrounding fundamental tissue. These trabeculae are the primordia of the lamellae. There is no annular gill cavity. The total depth of the zone occupied by the primordia of the lamellae and the pileus was 50  $\mu$ .

In globose specimens 1 mm. diameter (Plate X, figs. 3, 4) the stalk was clearly outlined, and occupied the greater part of the developing fungus. It was broadly conical, composed of vertical hyphae, the outer hyphae being (in part at least) continuous with the fundamental tissue. The pileus was well developed, its radial structure being clearly evident. The tissue between the pileus and the stalk formed, in longitudinal section, a narrow segment, almost the whole length of the stalk, and 0.25 mm. broad at the widest part. The bulbils were in process of development in this fundamental tissue. They arise at different points in the same vertical plane (Plate X, fig. 4). They do not originate in a continuous sheet which divides into bulbils later. In longitudinal sections along the plane of a bulbil, the cells of the latter appear rather loose at first. In sections, especially transverse sections of the fungus, which cut a bulbil at right angles, it is seen that many of them are hollow, and others show a line of division down the middle (Plate X, fig. 5). In the hollow bulbils, a palisade layer of short cells lines the cavity, while the others consist of two layers of palisade cells, in contact and united at their margins. Some developing bulbils are partly hollow and partly solid.

The bulbils are formed by short branches perpendicular to the vertical hyphae of the fundamental tissue. These branches arise close together, forming a short palisade tissue, completely enclosing a small area in a vertical radial plane. All the

branches are directed inwards towards that area. Consequently there is formed a layer of palisade tissue round a narrow cavity, or more usually a plate of tissue consisting of two opposed palisade layers, which merge into one another at their edges (Plate X, fig. 6). The cells of the palisade layer produce cuboid or oval, spore-like cells,  $2-3\mu$  diameter, at their apices, *i.e.* within the developing bulbil; these cells do not become free, but remain united in short chains, which, at least in the early stages, lie in line with their parent cells.

It would be expected that the bulbils arise in radial vertical planes of primordial tissue, in continuation of the more deeply staining primordia which descend into the fundamental tissue from the pileus primordium in the earlier stages of development. But when bulbil formation has begun, no deeply staining strands have been detected between the bulbils of a single lamellar series. Moreover, the fact that the bulbils are discontinuous in a lamellar series argues against the existence of a lamellar primordium continuous from the pileus downwards.

No longitudinal hyphae are present within the bulbils. They do not include any of the hyphae of the fundamental tissue. Consequently it would appear that the opposed horizontal branches arise from hyphae which lie close together.

The presence or absence of a cavity within the bulbil appears to depend merely on the number of palisade branches which arise round a given centre. From the general shape of the bulbils, the prior existence of cavities round which they might be formed appears improbable, and such cavities have not been observed. That the cavities in the bulbils are not artefacts would seem to be clear from their general shape and structure. The material was taken from culture and fixed in chrom-acetic acid. In any case, whether the cavity is artificial or not, it is quite clear that the bulbils are formed from without inwards.

There is no general curvature of the hyphae of the fundamental tissue into the bulbil. Some do curve towards the bulbil, but there is nothing resembling the general curvature of hyphae towards the hymenium seen in the trama of a developing gill.

Retaining the comparison with an agaric, we may visualise the development of *Rhacophyllus* as follows. In a normal agaric, the gill primordia develop into plates of tissue which produce a palisade layer of basidia on each side, *i.e.* externally, relatively to the primordial tissue. On the other hand, in *Rhacophyllus* the hypothetical gill primordium develops a palisade tissue internally from either side, and these cells unite into a solid tissue.

The bulbils increase in size, and, if hollow, become solid, by the addition of cells internally from the apices of the original

palisade cells, and by the enlargement of the individual cells. It is possible that in an early stage new branches from the fundamental tissue may penetrate between the cells of the original palisade layer. But they do not show any indication of the addition of cells externally.

With reference to the formation of bulbils discontinuously in the fundamental tissue, it is of interest to note that the hyphae of the fundamental tissue may form chains of spore-like cells at any point. In sections of the mature fungus, it is not uncommon to find spore-like cells lying in the interlamellar tissue. These, however, are best seen in sections of a specimen from which some of the bulbils have fallen (Plate X, fig. 7). The interlamellar hyphae which then extend from the pileus or stalk may bear short chains of thin-walled cells, either cuboid, about  $3\mu$  diameter, or oval,  $3 \times 2\mu$ . These arise laterally from the hyphae, and the chains may branch and form small clusters. But that the formation of the bulbils is not due to a haphazard production of chains of cells is evident from their occurrence in definite lamellar series.

The development of *Rhacophyllus* differs from that of the majority of the agarics which have been studied, in the absence of an annular gill cavity or furrow. But in this respect it agrees with *Amanitopsis vaginata* as described by Atkinson. In the latter there is no gill cavity, the primordia of the lamellae appearing first as trabeculae embedded in the fundamental tissue. In its further development, *Rhacophyllus* differs completely from *Amanitopsis vaginata*, as no interlamellar spaces are formed, and bulbils are produced instead of gills.

*Rhacophyllus* has been regarded as an agaric, in which the gills have been converted into bulbils. It will be evident that such a generalisation does not convey a correct impression. *Rhacophyllus* does not possess true gills, and the structures which occupy the position of the gills of an agaric are formed in a manner which may be said to be the reverse of that of a gill. In the latter respect, they agree more closely with the formation of the hymenium in the Lycoperdineae.

#### SYSTEMATIC.

The systematic position of *Rhacophyllus* is doubtful. In the possession of a universal veil, a definite radial pileus, and a stalk, composed of parallel hyphae, which lengthens at maturity, it is decidedly agaricoid; and its general appearance, both before and after expansion, is that of a *Psathyrella* or *Coprinus*. But none of these features is confined to the Agaricaceae. *Battarrea Steveni* (Tulostomataceae), as described by de Bary, has a universal veil, and its stalk, scarcely evident in the un-

expanded fungus, attains a length of 1 ft. on expansion. *Poly-plocium* and *Gyrophragmium* (Hymenogastrineae) have a central stalk, which elongates when the fungus is mature, and a volva; but whether the latter represents the remains of a universal veil or not does not appear to have been ascertained.

Another genus of the Hymenogastrineae, *Secotium*, has a similar stalk in some species. *Secotium agaricoides* (Czern.) Holl. has been recently studied by Conard, who found that the developing fungus possesses an outer layer homologous with the universal veil of an agaric, and is furnished with a gill cavity. The hymenium is borne on branched and folded anastomosing lamellae which grow downwards into the gill cavity, just as in the case of *Agaricus*, except that the lamellae are not plane. Consequently, Conard considers that *Secotium agaricoides* should be placed in the Agaricaceae, and that his results strengthen Fischer's opinion that the Secotiaceae should be dismembered and the genera distributed amongst the Hymenomycetes.

In *Secotium Mattirolianus* (Cav.) Fisch., the gleba in the unexpanded fungus does not show any lamellar arrangement in either longitudinal or cross section (cf. Fischer, in Engler-Prantl); it consists of branching and anastomosing folds, so that a section shows irregular cavities without any definite arrangement. *Secotium agaricoides*, according to Conard's photographs, presents the same appearance. Hence, if these can be included in the Agaricaceae, there would be little hesitation in similarly including *Rhacophyllum*, which has its bulbils in definite lamellar series, were it not for the structure of the bulbils.

The fact that the bulbils originate within the fundamental tissue, *i.e.* without the prior formation of a gill cavity, would not exclude *Rhacophyllum* from the Agaricaceae, since a similar condition occurs in *Amanitopsis vaginata*. But the mode of formation of the bulbils is opposed to any such reference.

If *Rhacophyllum* is considered to be derived from one of the Basidiomycetae, it can be compared, as regards the formation of the bulbils, only with species in which the basidia are formed as a lining to a small regular closed cavity. That rules out the Hysterangiaceae, in which the chambers of the gleba are formed by the fusion of trama plates, and most of, if not all, the Hymenogastraceae. It would appear that such a formation can be paralleled only on the Lycoperdineae or the Nidularineae, or, possibly, in some of the genera of the Plectobasidiineae. But the development of the majority of the species of the last-named group has not been investigated, and it is not certain that true hymenial cavities occur in any of them. As regards the presence of a stalk, it would be paralleled in that group by *Podaxon*.

In the absence of spores, it is perhaps natural to regard *Rhacophyllus* as an abnormal condition of some sporiferous fungus. But there is no evidence in support of that supposition, unless we accept the single specimen of a *Psathyra* which Duss forwarded with his specimens of *Rhacophyllus* from Guadeloupe, and in that instance there is nothing to indicate why Duss considered the two related. No normal agarics occurred with the Ceylon specimens, nor did any develop later from the material brought to the laboratory or in culture. *Rhacophyllus lilacinus* can be reproduced by means of the bulbils, and the latter are sclerotoid and consequently capable of surviving conditions adverse to growth. There is therefore no sufficient reason why *Rhacophyllus* should not be an autonomous fungus.

It would appear probable, from the description, that the specimens from Tonkin described by Patouillard were *Rhacophyllus lilacinus*. The specimens from Tunis, according to the description and figures, would appear to be another species, differing from the former in the shape of the bulbils.

No attempt has been made to repeat Moreau's cytological investigations. Material fixed in chrom-acetic acid is available.

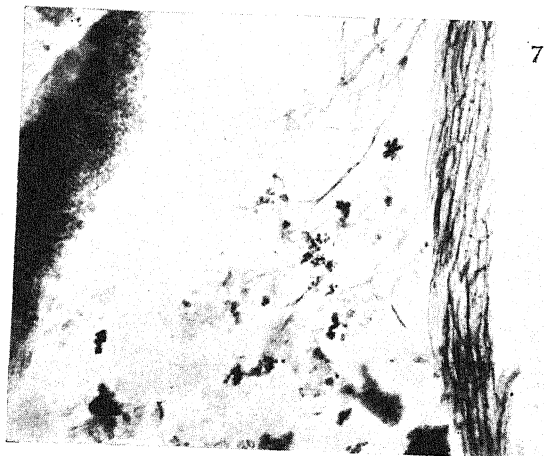
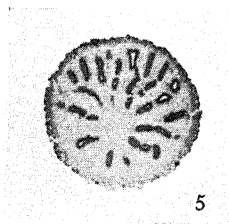
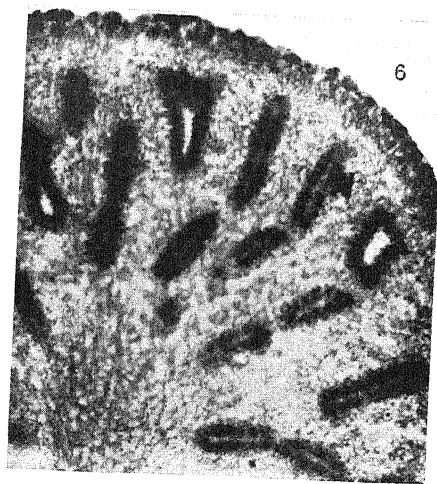
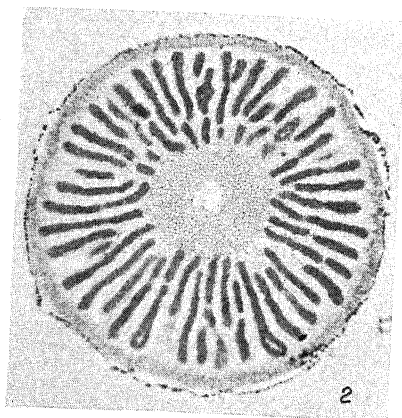
I am indebted to Mr L. S. Bertus for the slides and photographs.

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#### EXPLANATION OF PLATE X.

- Fig. 1. Longitudinal section of a fructification just prior to expansion.  $\times 15$ .  
 Fig. 2. Cross section of a fructification, 5 mm. high, shortly before expansion; the universal veil partly separated from the cap.  $\times 40$ .  
 Fig. 3. Median longitudinal section of a developing specimen, 1 mm. diameter, part of the universal veil detached in sectioning.  $\times 40$ .  
 Fig. 4. Tangential section of the same specimen as in fig. 3.  $\times 40$ .



RHACOPHYLLUS LILACINUS





Fig. 5. Cross section of an immature specimen, cut obliquely.  $\times 40$ .

Fig. 6. Part of the section in Fig. 5.  $\times 200$ .

Fig. 7. Part of a longitudinal section of a mature specimen from which some of the bulbils have fallen.  $\times 300$ . On the right, section of the cap, to which are attached strands of hyphae which bear chains of cubical cells in groups; on the left, part of a bulbil cut obliquely.

## STUDIES IN ENTOMOGENOUS FUNGI.

(With 1 Text-fig.)

### X. VERTICILLIUM SPP.

By T. Petch, B.A., B.Sc.

A SPECIES of *Verticillium* parasitic on *Aleyrodes* on *Citrus* was collected in Florida in 1905, and has since been found to be generally distributed in that state. It is not confined to *Aleyrodes*, but has occurred also on *Mytilaspis*, *Diaspis*, and *Lecanium*. The fungus was studied by Fawcett, who published a full account of it in his *Fungi parasitic upon Aleyrodes Citri* under the name *Verticillium heterocladium* Penzig. I have specimens of this species from Fawcett, and others from Webber, the latter in association with *Aegerita Webberi*. The first specimens recognised were also in association with *Aegerita Webberi*, but Fawcett has shown that the two are quite distinct.

The fungus is peculiar, as a *Verticillium*, in forming superficial stromata. In this respect it resembles *Aegerita Webberi*. The stromata are cinnamon brown, becoming ashy brown, flattened pulvinate, up to 2 mm. diameter, and 0.6 mm. thick. The surface of the mature stromata appears powdery, and when magnified is seen to be covered with free ends of hyphae. The stromata is compact, not spongy, and brownish yellow internally. There is usually a cavity at the base, up to 0.2 mm. high, in which the insect is situated. Each stroma covers a single insect, and, as in the Florida specimens of *Aegerita*, the scale persists beneath the stroma.

The hyphae of the stroma are 2-5  $\mu$  diameter, brownish yellow in the interior of the stroma, but hyaline towards the periphery. They are contorted and intertwined, but not fused into a parenchymatous tissue. The walls of the hyphae are thickened, so that the lumen is only about 1  $\mu$  diameter, but no completely solid hyphae have been observed. The septa persist, unthickened, in these thick-walled hyphae, and appear as narrow lines across the slender lumen. The contents of the hyphae stain with eosin, but the walls do not. As noted by Fawcett, the hyphae of the stroma tend to break up into short spore-like lengths.

The stromata are bordered by a rather coarse, byssoid or strigose margin, or hypothallus, about 1 mm. broad, which merges into a thin, whitish, byssoid or pulverulent film, extending indefinitely over the leaf. The hyphae of the hypothallus and the film are regular, hyaline,  $2-4\mu$  diameter, with a few stouter, brown,  $4-6\mu$  diameter. These hyphae are septate, and their walls are only slightly thickened. They sometimes bear sessile, oval, spore-like bodies,  $6 \times 4\mu$ , laterally, each just below a septum.

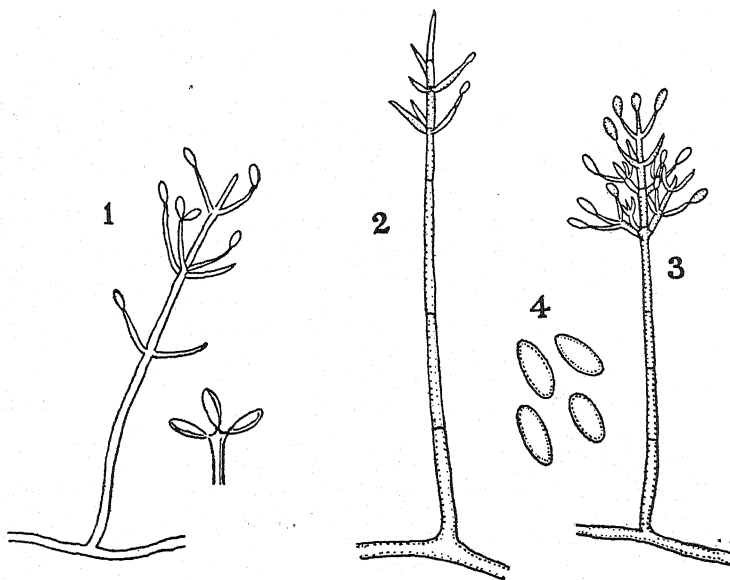


Fig. 1. *Verticillium heterocladium* Penz. 1, figure from *Fungi Italici* (magnification not stated); 2, young conidiophore,  $\times 450$  (Fawcett); 3, mature conidiophore,  $\times 450$  (Fawcett); 4, conidia,  $\times 1000$  (Fawcett).

The conidiophores arise from the hyphae of the film, or on the stromata. They are hyaline, erect, slender, septate, up to  $240\mu$  high (Fawcett), and  $2-3\mu$  diameter, with the basal segment sometimes inflated. At a considerable distance from the base, they bear either whorls of phialides, or whorls of branches which bear phialides at their apices. Usually there are two to four phialides or branches at a node. The lateral branches are more or less cylindrical,  $6-9\mu$  long and  $2\mu$  diameter. The phialides are elongated oval, attenuated above, somewhat inequilateral, curving upwards,  $6-12\mu$  long,  $2\mu$  diameter. The conidia are solitary, narrow oval, hyaline, and continuous. In

the specimens examined by me, they measured  $3.5-4 \times 1.5-2 \mu$ . Fawcett gives the dimensions  $4-6 \times 1.5-2.5 \mu$ .

Fawcett grew the fungus in pure culture on 5 per cent. glucose agar. The mycelium was at first white, with a reddish brown centre, ultimately becoming cinnamon-coloured and forming stromata. Conidia were produced at an early stage. In transfers to sweet potato and bread, the substratum became covered by a felted cinnamon-coloured stroma, while on rice and caladium stems the colours were ochre and brick-red respectively. The parasitism of the fungus was demonstrated by infecting larvae of *Aleyrodes citri* with spores from a pure culture.

This fungus has become generally known as the "cinnamon fungus." It has been recorded from the West Indies.

Fawcett referred this species to *Verticillium heterocladium* Penzig, a fungus which was originally found on *Lecanium hesperidum* on lemon leaves in Italy. It seems scarcely possible, however, that the identification can be correct. The original description of Penzig's species (*Michelia*, II, 462) is as follows:

*Verticillium heterocladium* Penzig. Hyphis repentibus, elongatis, paullum ramosis, continuis; ramis fertilibus adscendentibus, ramulosis; ramulis ternis vel quaternis, oppositis vel alternis, patentibus, rectis, apice attenuatis; conidiis in ramulorum apice solitariis, geminatis vel ternis, saepius pedicellis brevissimis suffultis, oblongis, hyalinis,  $5.5-6$  micr. long.,  $2-3$  micr. crassis.

It is to be noted that Penzig made no reference to any stroma, and that he stated that one to three conidia might occur at the apex of a phialide. Except for those points, the description might well be taken to fit the Florida species.

Penzig's figures were reproduced in Saccardo, *Fungi Italici*, Fig. 1193. These do not show anything resembling the characteristic stromata of the Florida species. The fungus covers the scale and forms a narrow white border round it, resembling, in that respect, *Cephalosporium Lecanii* and *Acrostalagmus coccidicola*. The figure of the conidiophore resembles that of the Florida species in the shape and position of the phialides, but in all cases the phialides are shown arising in whorls from the main stem, not from whorls of lateral branches. A more serious difference, however, is in the mode of attachment of the conidia. In the figure of the conidiophores the basidia are acute at the apex, with from one to three conidia, but in the enlarged figure of a phialide the apex is obtuse and three-toothed, each tooth bearing a single conidium.

One might suppose that the latter figure was based on an error of observation. But in the original description and in

*Studi botanici sugli Agrumi e sulle Piante affini* (1887), p. 398, Penzig added an explanatory note in which he stated that the distinctive character of the species was the marked variability of the arrangement of the fertile branches (*i.e.* phialides); sometimes these were verticillate in threes or fours, sometimes opposite, or sometimes solitary and alternate; in the ternate attachment (*inserzione*) of the conidia the species approached *Cladobotryum* Corda, and it ought perhaps to be separated from *Verticillium* on that character. Thus Penzig emphasised the mode of insertion of the conidia which he figured, and consequently it would appear that he could scarcely have made a mistake in his drawing.

Penzig stated that it was often found associated, on leaves of *Citrus*, with the species which he assigned to *Acrostalagmus albus* Preuss, from which it was distinguished by its mycelium and its external appearance.

In *Bull. Soc. Myc. France*, xxvii (1911), 486, Fron recorded *Verticillium heterocladium* Penzig on chrysalides of *Cochylis ambiguella* in France. He stated that it was easily cultivated and showed a characteristic arrangement of the hyphae. The hyphae bore numerous branches, sometimes isolated, sometimes three to five in a whorl. His figures show a striking resemblance to *Cephalosporium* (*Acrostalagmus*). That of the general habit of the fungus would quite well represent the repent hyphae of *Cephalosporium*, while the conidiophores show exactly the arrangement and variation in number of the phialides which obtains in the entomogenous species of *Cephalosporium*. Only a single conidium is shown at the apex of each phialide.

Fron did not give particulars of his cultures, and it would appear that he did not observe colour changes such as those recorded by Fawcett. Moreover, his figures do not agree with the American species, nor does his fungus agree with that described by Penzig, who laid special emphasis on the occurrence of one to three conidia at the apex of each phialide.

I have not been able to examine European specimens of *Verticillium heterocladium*, and consequently cannot carry this investigation further.

## STUDIES IN ENTOMOGENOUS FUNGI.

(With 1 Text-fig.)

### XI. EMPUSA LECANII ZIMM.

By T. Petch, B.A., B.Sc.

IN "De dierlijke Vijanden der Koffiecultuur op Java," Deel. II (*Meded. wit's Lands Plantentuin*, XLIV, 1901), Zimmermann described (pp. 25-27) a fungus, *Empusa Lecanii*, which attacked

the green bug (*Lecanium viride*) in Java. He styled it the "Black Scale-insect Fungus."

The affected insects could generally be recognised by their colour, the green scale becoming white. They next turned greyish, and then darker and darker until they were almost black.

Under the microscope it was seen that in the white stage the body of the insect was filled with colourless spherical cells, which were present even in the legs and antennae. These were densely crowded, but towards the margin of the body they might be isolated and it could then be determined that they were not connected with any hyphae.

When the insect had become grey, it was found that the spherical cells in the middle of the body had germinated, giving rise to short stout hyphae (basidia), each of which bore at the apex a pyriform conidium,  $18 \times 9-10 \mu$ . From Zimmermann's figures it would appear that the basidia were about  $55 \mu$  high, and  $7-9 \mu$  in diameter. The wall of the conidium was dark coloured, the dark colour of the diseased insect being due to the colour of the conidia. The conidia often germinated on the host; Zimmermann's figures show a conidium with three germ tubes.

Zimmermann stated that it seemed that the fungus might best be placed provisionally in the genus *Empusa*, although it was exceptional in that genus on account of the dark colour of the conidia and their germination by a germ tube.

The fungus was again recorded in 1918 by Coleman and Kunhi Kannan in "Some Scale Insect Pests of Coffee in South India" (*Dept. of Agriculture, Mysore State, Ento. Series, Bull. No. 4*). The early stages of its attack were shown by a whitish discoloration of the insect, but later it turned to a dark grey, while a greyish growth of fungus hyphae appeared on its surface. The conidia were oval. If the body of an affected insect was cut open, a mass of dark brown, spherical bodies was found inside. The fungus was not noticeable on coffee estates during the south-west monsoon, but occurred during the lighter north-east monsoon and during the early part of the drier cold weather. Under favourable circumstances, it might be found active until May, i.e. through the dry weather and almost up to the time of the heavy rains of the south-west monsoon.

Dr Coleman has kindly forwarded me specimens of the Indian species on *Lecanium viride* on guava, and I have similar specimens on *Lecanium viride* on coffee, Kotagiri, Nilgiris, per Mr R. D. Anstead, August, 1910. I have also similar specimens from Ceylon on *Lecanium viride* on *Futumia*, Peradeniya, October and November, 1913, and on a scale on *Cinnamomum ovalifolium*, Hakgala, February 27th, 1922.

In none of these specimens have I been able to find any *Empusa*. When the scale is in the white stage, it contains a very fine hyaline mycelium,  $0.5-1\ \mu$  diameter, usually sparsely distributed. One or two hyphae may extend from the scale over the leaf and may bear a pyriform conidium at the tip. Numerous scattered spores of Dematiace, e.g. *Cladosporium*, etc., are usually present; and the scale may bear small patches, consisting of a few cells coalescent in one plane, which belong to the same fungi.

When the scale has become grey, it may be completely covered with the pyriform conidia. The fine mycelium is then abundant, but the hyphae remain separate, and do not fuse into masses. Hyphae and conidiophores of *Cladosporium* are usually present.

In general, however, when the scale darkens, the insect is permeated by the brown, irregular hyphae of one or more Dematiace. Moniliform chains of cells and small plate-like clusters are common, and conidiophores of *Cladosporium*, etc., arise at the margin. The fine hyaline mycelium with its pyriform conidia is present, but it is overgrown by the Dematiace.

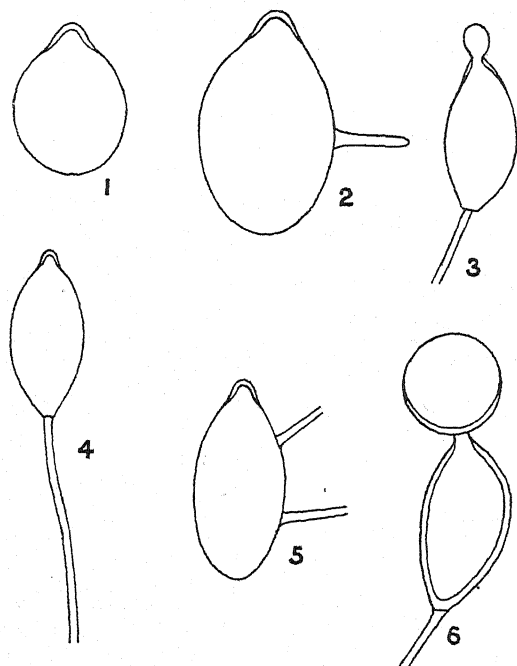
Finally, the whole scale is covered with a dense growth of conidiophores. In the Ceylon specimens these are for the most part *Cladosporium*, but in the specimen on guava from South India, a *Macrosporium* is the chief constituent. The scale at this stage is merely a thin film adherent to the leaf.

Thus, while the conidia of the hyaline mycelium become fuscous or pale brown, and might conceivably cause the scale to appear black, the black colour in all the available specimens is due to a dense growth of Dematiace on the dead scale.

The hyaline mycelium is very fine,  $0.5-1\ \mu$  diameter, regular and not septate. The conidia are borne singly on free hyphae, or conidiophores, up to  $60\ \mu$  long,  $0.5-0.75\ \mu$  diameter, arising at the margin of the scale or anywhere on its surface. I have not seen a branched conidiophore. The conidia are narrow-oval or pyriform, with a small but well-marked papilla, and measure  $12-20 \times 5-10\ \mu$  (Fig. 4). A few may be broader and more globose,  $12 \times 9\ \mu$  (Fig. 1). They are thin-walled, except at the papilla. The scar of attachment is usually well-defined, and broader than the conidiophore. At first the conidia are hyaline, but they ultimately become fuscous or pale brown. These conidia are exactly those of a *Pythium* or a *Phytophthora*.

In general, the conidia germinate by the production of a germ tube, sometimes two, laterally (Figs. 2 and 5). The germ tubes are of the same diameter as the mycelium. In one collection, however, on *Lecanium viride* on *Futumia*, Peradeniya, November 5th, 1913, the papilla in very many cases has burst and the contents of the conidium (sporangium) have partly emerged

into a globose vesicle (Fig. 3). In one instance (Fig. 6) this vesicle forms a spherical spore-like body,  $9\mu$  diameter, which appears to be persistent; the wall of this is thickened on the lower side. In some instances, there is a small swelling on the conidiophore a short distance below the conidium, but I have not observed any further development of this.



Figs. 1 and 4, sporangia; figs. 2 and 5, sporangia germinating; figs. 3 and 6, sporangia with vesicle. All  $\times 1600$ .

The hyaline, globose spores noted by Zimmermann as occurring in the body and legs of the insect are not abundant in the available specimens. They have been observed in the specimen on *Futurnia* noted above. They measure  $8-10\mu$  in diameter, and are apparently attached to a very fine hyaline mycelium, similar to that which bears the oval or pyriform conidia.

In one collection, on a black *Aleyrodes* on *Cinnamomum ovalifolium*, the conidia are inequilateral. The mycelium is of the same dimensions as that described above.

Attempts to get this species into culture have failed, owing to the difficulty of separating it from bacteria and Dematiace. On the characters observed it would appear that it should be included in the genus *Pythium*. But it is aberrant in its fuscous



or pale brown spore wall, and in the formation of a persistent vesicle.

As I have not seen the type specimen of the fungus described by Zimmermann as *Empusa Lecanii*, it is not possible to make any definite statement concerning it. The recorded effect of *Empusa Lecanii* on its host is similar to that of the present fungus, and the conidia of the former, as described by Zimmermann, resemble those of the latter species in shape, size, and colour. Moreover, Zimmermann stated that the conidia of his fungus germinated with several germ-tubes, as in the present species. Consequently, it would appear that there is a strong probability that *Empusa Lecanii* is identical with the fungus described in this paper.

The correct position of this fungus can, however, only be decided by growing it in pure culture.

## ENTOMOGENOUS FUNGI.

### ADDITIONS AND CORRECTIONS, II.

(With 1 Text-fig.)

By T. Petch, B.A., B.Sc.

#### *Moniliopsis rigida* Petch, n.sp.

White to pale yellow, forming a loose, but somewhat rigid crust of interwoven hyphae, overlying colonies of mites. Hyphae regular, hyaline,  $5\ \mu$  diameter, but varying in different lengths from  $5$  to  $8\ \mu$ , bearing chains of spore-like cells terminally and laterally. Lateral chains simple, of 3–5 cells, arising either from a normal segment of the hypha, without any conidiophore, or from an intercalary polygonal cell which more or less resembles the cells of the chain; terminal chains of up to 6 cells, branching. Pseudoconidia or spore-like cells oval, truncate at both ends (except the terminal one of the chain), attached by a broad base, increasing in size towards the apex of the chain,  $18\text{--}28 \times 16\text{--}17\ \mu$ , not separating; wall stout,  $1.5\ \mu$  thick.

On mites on *Phyllostachys bambusoides*, Brooksville, Florida, February, 1924, per J. A. Stevenson.

#### *Rhinotrichum album* Petch, n.sp.

White; conidiophores up to  $0.1$  mm. high,  $1.5\text{--}2\ \mu$  diameter, rigid, erect, simple, or once dichotomously branched, at first in minute loose clusters, ultimately covering the insect with a loose white pile and spreading over the leaf; conidiophores hyaline,

rather closely septate, equal, slightly broader ( $2.5\ \mu$ ) and clavate at the apex, bearing conidia everywhere on short sterigmata about  $1\ \mu$  long; conidia globose or slightly oval,  $1.5$ – $2\ \mu$  diameter, hyaline, minutely verrucose.

On *Lecanium hemisphaericum*, Hakgala, March, 1922.

***Cephalosporium falcatum* Petch, n.sp.**

Hyphae scanty, hyaline,  $3\ \mu$  diameter: conidiophores in whorls of three, up to  $26\ \mu$  high, inflated at the base to  $2\ \mu$  diameter, strongly attenuated from half their height to the apex, heads oval, about  $12 \times 6\ \mu$ ; conidia falcate, tips attenuated and acute, or narrow fusoid with the ends produced, straight or curved, continuous,  $10$ – $13 \times 1.5$ – $2\ \mu$ .

On a fly attached by the mycelium to the under side of a living leaf, Hakgala, March, 1922.

***Coremium pulcherrimum* Petch, n.sp.**

Mycelium pinkish red, forming a network over the insect, sometimes uniting into strands. Synnemata arising at the margin of the scale or from the mycelium on its surface, up to 2 mm. high; stalk 0.4 mm. diameter, pinkish red, longitudinally fibrillose and rather loose, composed of hyphae  $2\ \mu$  diameter, which separate above to form long conidiophores; head irregularly ovoid, loose, white, up to 1 mm. high, 0.9 mm. diameter; conidiophores long,  $2\ \mu$  diameter, bearing dense whorls of phialides, about  $20\ \mu$  apart on the uppermost 20 to  $50\ \mu$ ; phialides flask-shaped,  $5\ \mu$  long,  $2\ \mu$  diameter below; conidia catenulate, hyaline, oval,  $2 \times 1.5\ \mu$ , or globose,  $2\ \mu$  diameter.

On *Lecanium nigrum*, etc., Peradeniya, August, 1921.

This species occurred on specimens of *Lecanium nigrum*, attacked by a parasitic insect, which were kept in glass tubes for the emergence of the parasite. The fungus attacked both the *Lecanium* and its parasite, developing on the latter after its emergence from the scale.

***Coremium breve* Petch, n.sp.**

Synnemata up to 0.9 mm. high, with a stout, yellow, glabrous stalk, 0.4 mm. high, 0.25 mm. diameter, which expands into a white, loose, laterally compressed, circular head, 0.5 mm. diameter; some examples sessile, pulvinate, forming white powdery tufts, 1 mm. long, 0.6 mm. broad, at the margin of the scale. Conidiophores  $2\ \mu$  diameter, branched, with lateral branches up to  $30\ \mu$  long; heads of conidia terminal, the

conidiophores bearing short lateral branches just below the apex, which are crowned by flask-shaped phialides,  $6\ \mu$  high,  $2\ \mu$  diameter, forming a closely crowded head; conidia narrow-oval, hyaline, ends acute,  $1.5-2 \times 1\ \mu$ .

On *Lecanium* sp., Hakgala, March, 1922.

***Coremium gracile* Petch, n.sp.**

White, clavate, shortly stalked, sometimes branched, up to 1.5 mm. high, 0.4 mm. diameter below, with an irregularly ovoid head, up to 0.7 mm. diameter; stalk loose, longitudinally fibrillose, composed of hyphae  $3\ \mu$  diameter; conidiophores branched above, with two or three stout branches,  $10\ \mu$  long, in a whorl; branches bearing whorls of stout subcylindric phialides,  $6 \times 3\ \mu$ , slightly inflated at each end, which bear flask-shaped phialides,  $7 \times 2\ \mu$ ; conidia hyaline, oval, one end acute,  $1.5-2.5 \times 1-1.5\ \mu$ , or subglobose,  $1-1.5\ \mu$  diameter; heads of conidiophores up to  $50\ \mu$  high,  $40\ \mu$  diameter.

On a spider enclosed in a silk web, the coremia developing on the web, Peradeniya, July, 1921; Hakgala, March, 1922.

***Fusarium entomophilum* Petch, n.sp.**

Mycelium white, sparse, overrunning the insect, and bearing purplish pulvinate acervuli, up to 1 mm. diameter: acervuli violet by transmitted light when fresh; conidia fusoid, ends falcate, or almost straight, four or five septate,  $36-52 \times 4-5\ \mu$ .

On *Clitellaria heminopla*, Suduganga, April 12th, 1919 (coll. R. Senior White).

***Stilbum (Stilbella) coccorum* Petch, n.sp.**

Synnemata scattered, white, up to 0.3 mm. high,  $65\ \mu$  diameter below, equal, or expanding above into a flattened-globose head up to  $160\ \mu$  diameter; stalk minutely tomentose, sometimes forked above; conidia hyaline, globose,  $1.5\ \mu$  diameter, or oval,  $2 \times 1.5\ \mu$ .

On *Fiorinia juniperi* on *Juniperus bermudiana*, Peradeniya, December 1923.

The synnemata arise at the margin of the scale, no mycelium being visible externally, but the scale is filled with white mycelium.

A species of *Stilbum*, *S. coccophilum*, was described by Saccardo (*Ann. Myc.* v, p. 178, 1907) on *Ceroplastes rusci* in Sicily. According to the description, the scale is covered with a mycelium from which the synnemata arise. The synnemata are up to 0.9 mm. high, filiform, with a glabrous stalk,  $40\ \mu$  diameter, and a globose, glabrous head,  $200\ \mu$  diameter. The head is not notably

attenuated into the stalk. The conidia are oblongo-fusoid, hyaline, somewhat acute,  $6.5-7 \times 2.5 \mu$ .

I have not seen the type of *Stilbum coccophilum*, but it appears from the description to be quite distinct from the Ceylon species.

***Hirsutella nodulosa* Petch, n.sp.**

Mycelium loose, grey; hyphae hyaline to pale brown, regular, septate, rather rigid, wall stout; basidia lateral, rarely terminal, simple, subcylindric, conoid, or flask-shaped, up to  $16 \mu$  high,

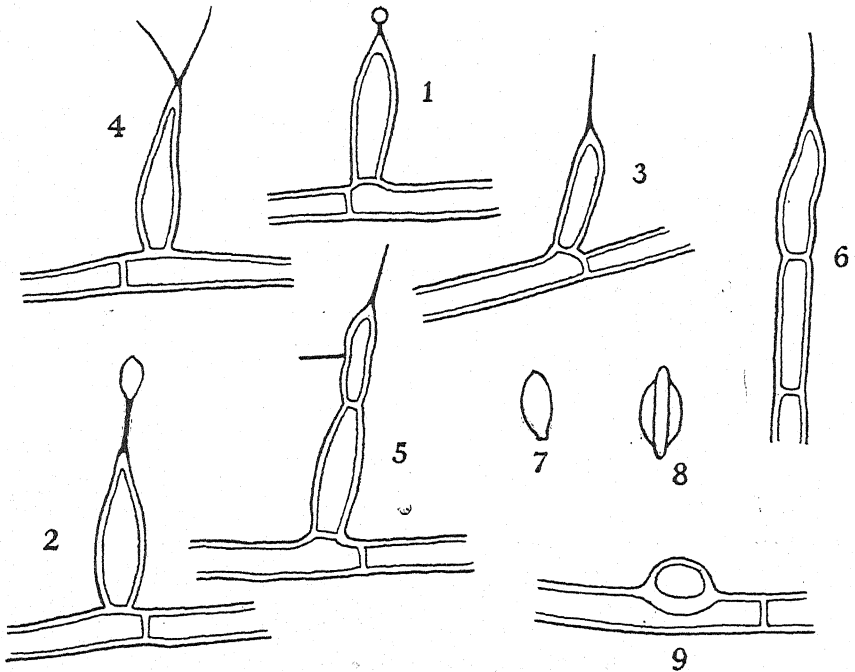


Fig. 1. *Hirsutella nodulosa* Petch. 1, 2, basidia with developing conidia; 3, basidium and sterigma; 4, 5, abnormal basidia; 6, a terminal basidium; 7, a single conidium; 8, a cluster of conidia; 9, swelling on a hypha. All  $\times 1200$ .

$3-4 \mu$  diameter; sterigmata usually terminal and solitary, up to  $16 \mu$  high,  $1 \mu$  diameter, minutely nodular; spore clusters broadly oval, apiculate,  $9 \times 6 \mu$ ; individual conidia oval, apex subacute, base truncate apiculate,  $6-7 \times 3 \mu$ .

On caterpillar of *Zeuzera coffeae*, Peradeniya, March, 1925.

*Zeuzera coffeae* is the Red Borer of coffee, tea, etc. In this instance, the caterpillar was found dead in its gallery in a twig of mahogany. From the nodular appearance of the longer

sterigmata, it would appear that the spore clusters are produced in succession on the one sterigma. Occasionally two sterigmata occur at the apex of a basidium, and sometimes one laterally in addition to the normal terminal sterigma. More rarely a basidium may be septate, or may give rise to another basidium laterally. Hemispherical protuberances occur commonly on the hyphae; these are subglobose or ovoid cells, the inner wall of which is strongly thickened and projects into the hypha.

Only one specimen of this species is available, and this differs from the type of *Hirsutella* in not possessing a clava.

***Acremonium griseum* Petch, n.sp.**

Grey, or slightly lavender, forming a loose, pulverulent, floccose mass: hyphae hyaline, regular, thin-walled,  $1-1.5 \mu$  diameter; conidiophores lateral, simple, elongated conical or flask-shaped, up to  $10 \mu$  high,  $1.5 \mu$  diameter below; conidia terminal, hyaline, continuous, narrow-oval,  $3-4 \times 1-1.5 \mu$ .

On spiders attacked by *Hirsutella* (?), Hakgala, March, 1922.

The insect is covered with grey or lavender floccose masses, and the mycelium spreads from it over the leaf, forming powdery patches covered with minute tufts. At first sight, the fungus appears to be parasitic on the insect, but further examination shows that one of the apparent legs of the insect is a decumbent clava, about  $0.1$  mm. diameter, which appears to be an abortive clava of a *Hirsutella*. The fungus is, as far as can be ascertained, parasitic on the *Hirsutella*, not on the insect.

***Sporotrichum album* Petch, n.sp.**

Conidiophores suberect, arising from the repent mycelium in minute tufts, afterwards decumbent in white floccose masses, up to  $50 \mu$  long, simple or once branched,  $1.2 \mu$  diameter, somewhat geniculate above, widening at the angles to a breadth of  $2 \mu$ ; conidia terminal or lateral, in the latter case sessile on the upper face of the angular projection, solitary, hyaline, continuous, cylindric, rounded above, subacute at the base,  $6-10 \times 1.2-1.5 \mu$ .

Parasitic on *Cordyceps dipterigena* B. & Br. on *Mydaea* sp., Hakgala, March, 1922.

The fungus may be confined to the head of the *Cordyceps* or may cover the whole of the clava with a white floccose mass. It also spreads over the insect, and appears on the anterior margins of the wings; but as the insect is permeated by the hyphae of the *Cordyceps*, it is no doubt parasitic on the *Cordyceps* wherever it appears.

***Tubercularia epimyces* Petch, n.sp.**

Sporodochia black, minute, pulvinate, gregarious or confluent, parenchymatous below, bearing closely-packed conidiophores; conidiophores with a simple base about  $8\mu$  long,  $2\mu$  diameter, branching above; branches about  $6\mu$  long, bearing densely clustered, flask-shaped basidia,  $8\mu$  long, at their apices; conidia hyaline, oval,  $2 \times 1\mu$ .

On *Aegerita Webberi* Fawcett on a scale insect on *Psychotria*, Hakgala, March, 1922.

The *Aegerita* is blackened, and from it fuscous hyphae radiate in a film over the leaf. These hyphae are  $2\mu$  diameter, thin-walled, slightly irregular, and are united into strands. Here and there they bear blackish fuscous masses, about  $50\mu$  diameter, which bristle with irregularly conical fascicles of hyphae, or single hyphae, up to  $30\mu$  high. These masses may be developing pycnidia. The fungus has also been found on stromata of *Torrubiella*, Hakgala, September, 1923 (cf. p. 66).

***Melanosphaeria circumdata* Saw.**

This new genus and species were described by Sawada in *Descriptive Catalogue of Formosan Fungi*, II, December, 1922. The description is in Japanese, but makes reference to *Aspidiotus fici* and *Microcera Fujikuroi*. From the figure, the species appears to be one of the black pycnidial fungi which are parasitic on entomogenous fungi, viz. either *Sirosphaera* (1913) or *Sirosperma* (1916).

***Lycogala fragilis* Holm.**

This species, which occurred on a cockchafer (*Scarabaeus Melolontha*) was described by Holm in *Vid. Selsk. Skrifter*, Nye Samling, No. 1, p. 288, with fig. 5 (Kbh. 1781). Cooke, in *Vegetable Wasps and Plant Worms*, p. 108, wrote, "Nothing can be said of the so-called *Lycogala fragilis*, which was stated by Holm to have occurred on a broken and decayed specimen of *Melolontha vulgaris*, infesting the sides of the body and legs with small round spots. It was certainly not a *Lycogala*, but it may have been a *Laboulbenia*, or even *Leocarpus vernicosus*." As regards the insect, Holm merely stated that it was dead. From the description and figure there is little doubt that the fungus was a *Beauveria*.

***Botrytis necans* Massee.**

This was described by Massee in *Kew Bulletin*, 1914, p. 159, from specimens on larvae of *Brachartona catoxantha* from Singapore. There is no type or other specimen in Herb. Kew.

***Botrytis Rileyi* Farlow.**

This species was described by Farlow in the *Report of the (United States) Commissioner of Agriculture for the year 1883*, p. 121. It escaped inclusion in Saccardo, *Sylloge Fungorum*, and consequently has been unknown to mycologists in other countries, except through incidental mention, usually in entomological papers. During recent years it has been recorded on several occasions in the United States; and Dearness, in *Mycologia*, xvi (1924), p. 173, stated that it killed thousands of larvae of *Plathyrena scabra* on beans in 1922. For convenience of reference, the original description may be quoted here.

"*Botrytis Rileyi* Farlow. Mycelium hyaline, diffusely branched,  $1.5-2.5 \mu$  in diameter. Spores in whorls, which are approximate at the ends of the hyphae and remote lower down. Whorls formed at the base of elliptical cells attached rather obliquely to the axis, developing into chains of, at first oval, and at length nearly spherical spores,  $2-3 \times 1.5-2 \mu$ ; when young whitish, becoming verdigris-green when mature. On larvae of *Plusia brassicae*, covering them with a distinctly green powder. This species is related to *Botrytis bassiana* Bals., which attacks silkworms, and from which the present species can easily be distinguished by the green-coloured spores. The chains of spores, although in general resembling those of *B. bassiana*, are more fully developed than in any form of that species."

This species would be exceptional in the *Beauveria* (*Botrytis*) *Bassiana* group in respect of its colour, and the description does not suggest *Beauveria*. If commas are inserted after the words "formed" and "base," the description agrees well with that of a *Spicaria*. The type specimen should be compared with *Spicaria prasina* (Maubl.) Saw. Watson (*Rept. Fla. Agric. Dept.*, 1915, p. xli) states that in Florida the caterpillar of *Anticarsia gemmatilis* on velvet bean is attacked by *Botrytis Rileyi*, the larvae turning first grey and then white. Specimens of a fungus on this caterpillar on the same host plant, sent to me from Florida, are green, and the fungus is *Spicaria prasina*.

***Isaria stellata* Cke.**

This species was described by Cooke from specimens on mango leaves from Mysore, the description in *Grevillea*, iv (1876), 116, being "Nivea, stellata, incrustans. Floccis tenuissimis, circinatis. Encrusting dead insects attached to the under surface of mango leaves. Mysore. Snow white, encrusting minute insects and assuming the appearance of stellate bodies about 1 line in width. Threads very delicate, circinate, sigmoid or variedly curved. (Spores not seen.)"

In a *Report on Diseased Leaves of Coffee and Other Plants* (1876), Cooke wrote: "Specimens No. 4 of 'Mango leaves affected by a peculiar blight,' exhibited two forms of fungi, one black, occupying a large portion of both sides of the leaves, and the other of a snowy whiteness confined to the centre of the leaves on either side the midrib, on the under surface. The latter fungus in no way affects the tree, since it is not a parasite of the leaf itself, but was found investing minute dead insects attached to the leaf. These insects were so completely enveloped and metamorphosed by the snowy white woolly fungus (*Isaria stellata* Cooke, in *Grevillea*, iv) in which they were imbedded, that their precise character could not be ascertained, but probably they were some species of aphids, which is found to delight in Mango trees."

Cooke gave a further account of this species in his *Vegetable Wasps and Plant Worms*, pp. 306, 307. "Some Mango-leaves from India came into our hands some years since, and upon the under surface were found beautiful star-like, snow-white objects, almost like crystals of snow. Minute examination proved them to be minute insects, apparently *Aphides*, encrusted with an *Isaria*, about two millimetres in diameter, and sometimes confluent. The rays were numerous, radiating from a sort of discoid centre, regular, and not at all corresponding to any members, or projections, of the embedded insects; the latter impossible to extricate for identification. The whole mass of fungus was composed of delicate agglutinated threads. As often is the case, a considerable number of *Aphides* were congregated on the leaves, so that when they were close together, the encrusting *Isaria* united, becoming confluent in an irregular crust: but in cases where the insects were isolated, the star-like shape of the parasite was maintained."

The type in Herb. Kew is marked "on aphides." It consists of a group of insects which are furnished with waxy processes, such as commonly occur on leaves in the tropics, on which they form white patches. The type of *Isaria stellata* consists of the usual white, confused mass of insects and loose processes, with isolated insects which bear straight horizontal processes radiating from the margin of the insect over the leaf. The insect is *Phenacoccus mangiferae* Green. There is no *Isaria*. Apparently Cooke mistook the processes for a fungus.

### *Oospora Aphidis* Cke & Massee.

This species was described by Cooke and Massee from specimens sent by Bailey from Australia. It was said to have catenulate, lemon-shaped, apiculate conidia,  $17-19 \times 12 \mu$ .

In *Vegetable Wasps and Plant Worms*, p. 314, Cooke stated:



"Pumpkin-leaves, the under surface of which were attacked by Aphides, were sent to us from Queensland for examination, and we found that the dead insects were covered with a minute mould, having but very short and almost inconspicuous threads, but a profusion of spores, produced regularly in chains. In form they had resemblance to a lemon with an apiculus at each end, containing nuclei at first, but without colour, from seventeen to nineteen micro-millimetres in length, and twelve in breadth. The same mould did not appear on the leaves, apart from the insects, so that it is evident it did not spread from the plant to the dead insects (plate 2, fig. 21). At first there might seem a suspicion of this, since the species of *Oidium* and *Oospora* are common, and injurious to the foliage of living plants."

Bailey sent the specimen because it bore an *Oidium*. The mycelium of the *Oidium* covers the leaf, and bears typical conidia,  $28-40 \times 15-18 \mu$ , sometimes in chains. The aphides bear scattered conidia, either globose,  $15-16 \mu$  diameter, or oval,  $16-18 \times 12-15 \mu$ , broadly apiculate; these are the conidia of an *Entomophthora*. Cooke and Masee's measurement was probably based on the conidia of the *Entomophthora*, but the figure in Cooke's *Vegetable Wasps and Plant Worms*, which shows catenulate conidia, was more probably derived from the *Oidium*. The name *Oospora Aphidis* is to be discarded.

## ON A NEW SPECIES OF UROPHLYCTIS PRODUCING GALLS ON LOTUS CORNICULATUS LINN.

(With 26 figs. arranged as Plates XI—XIV.)

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IN the summer of 1921 my attention was directed by Professor M. C. Potter to some peculiar gall-like structures of fungus origin, which he had observed about eighteen years previously on the bird's-foot trefoil (*Lotus corniculatus* Linn.). A search for these in the field where they were originally discovered proved fruitless, but they were found in another field, distant about half a mile. Even here the galls were by no means plentiful, and to obtain specimens of them a careful and thorough search was required. About the same year in which he first found the galls, Professor Potter discovered another locality for them in a field separated from the previous station by a distance of about

thirty miles. We visited this spot together recently, but were unsuccessful in procuring any specimens. A careful and diligent search in other fields where *Lotus corniculatus* grows has not so far yielded any examples.

Thus, at the present time, only one locality is known for these galls, and this is situated at about eleven miles nearly due north-west of Newcastle-upon-Tyne. The infected plants of bird's-foot trefoil grow in the lowest and dampest part of a pasture field.

#### DESCRIPTION OF THE GALLS.

The galls occur usually in the region of the "collar," so that they are situated just above the surface of the soil, but occasionally they are found upon the creeping underground stems. A shoot seldom bears more than one or two, but sometimes three, or even four, of them are found together, and they are usually situated laterally upon the stems. They have a more or less spherical or ovoidal form, and are rarely lobed. The situation and appearance of the galls is shown in Fig. 1. The largest galls measure about 1 cm. in the longest diameter, but the average size is that of a fair-sized garden pea. On the underground stems some very small ones were found, about 1 mm. in diameter, and bearing some resemblance to root-nodules.

The colour of the galls, when freshly obtained, is a pale brown; the surface is smooth and slightly scurfy. The very young ones are of a pale yellow colour.

The gall-bearing plants appear to be as healthy and to reach the same size as ordinary individuals. They produce no flowers, but this is usually the case with plants of *Lotus corniculatus* when growing in rather moist situations. Plants of *Lotus uliginosus* Schk. which grew in the same spot, were entirely free from galls.

The cut surface of a gall has a white and brown mottled appearance. A section cut by hand and examined under the microscope discloses a parenchymatous tissue consisting for the most part of thin-walled cells, and numerous rounded cavities of various shapes and sizes mostly filled with brown resting sporangia (Figs. 2-4). The hemispherical form of the latter, the scarcity or almost entire absence of hyphae, as well as the general structure of the gall, are all characteristic of the Chytridiaceous genus, *Urophlyctis*, of which this is apparently a new species.

#### METHODS.

Small pieces of the galls were fixed in Carnoy's fixing fluid, in corrosive sublimate acetic acid alcohol, in medium chrom-acetic solution, and in Flemming. The microtome sections were stained with diamant fuchsin and light green, or with Haiden-

hain's iron-alum method, using orange G in clove oil as a contrast stain, or with Flemming's triple stain. The best results were obtained with the first two fixing media followed by Haidenhain's iron-alum method. Hand-cut sections stained with cyanin and orange G in clove oil were also very useful, as well as preparations made by teasing up the fresh galls in water on a slide.

#### MICROSCOPIC STRUCTURE OF THE GALL.

The gall is composed principally of thin-walled parenchymatous cells containing a nucleus and abundant starch grains; irregular vascular bundles, in which short tracheids with transverse pits are conspicuous, traverse the gall in various directions. There is no definite epidermis or any signs of the formation of a periderm, but the outermost cells are mostly empty and dead.

The cavities containing the resting sporangia (Figs. 2 and 3) occur sometimes apparently isolated, sometimes in groups, the separate chambers being mostly in open communication with one another, or separated only by intervening cell walls. I think it probable, however, that the apparently isolated chambers are linked up with others situated either above or below, so that the system of cavities forms one connected whole.

The cavities show a considerable diversity in form and size, but their contour is usually more or less rounded. The walls by which they are bounded are thicker and more distinct than the ordinary cell walls (Figs. 16 and 17). The cavities themselves appear to be formed by the breaking down and digestion of the walls of cells, the remains of the protoplasm and nuclei of which (Figs. 16 and 17, *n.*) are often to be seen between or around the immature resting sporangia.

#### THE RESTING SPORANGIA.

A mature resting sporangium has the form of a hemisphere with a slightly convex base (Figs. 5 and 20), but after drying or immersion in spirit the base becomes concave, thus agreeing with the descriptions and figures of other species of *Urophlyctis*. Attached to the centre of the base of the sporangium by a very short stalk is a small transparent vesicle or cell (not shown in Figs. 5 or 20), with a very fine hypha springing from the pole opposite to the sporangium. A resting sporangium has a diameter of 40–50  $\mu$  and a height of 25–30  $\mu$ . The wall is of uniform thickness (4  $\mu$ ) except at the angle formed by the meeting of the two curved surfaces where its thickness is increased; it is of a pale yellowish brown colour. In it two layers can be distinguished, a thick, coloured exospore, and a thin, colourless endospore (Fig. 21). The exospore is hard and brittle, almost glassy

in texture, so that in microtome sections it is frequently found broken into irregularly shaped pieces.

The more convex surface of the resting sporangium is ornamented with an irregular pattern of conspicuous grooves (Figs. 5, 6 and 14), varying in form, length and width, but their principal direction is from the apex downwards. The base is finely granulate, with a small pit in the centre, marking the spot where the colourless vesicle is attached.

The immature resting sporangia, which may be recognised by their paler colour and less conspicuous markings, have the apex smooth, and when boiled in caustic potash and afterwards cleared in chloral hydrate, show when examined under a one-twelfth inch oil immersion objective a rather irregular ring of very fine pores surrounding the smooth apex (Fig. 6). The number of these varies from five to nine, and is most usually six. It is almost impossible to distinguish these pores on the mature resting sporangium, on account of the surface ornamentation which covers the apex. Through each pore passes a small, branched, haustorium-like process (Figs. 7 and 10-13) while the sporangium is growing, but these processes disappear when the sporangium has reached maturity.

From the examination of the resting sporangium of a number of species of both *Urophlyctis* and the closely related genus, *Physoderma*, Jones and Drechsler<sup>(9)</sup> concluded that a certain range in the number of these pores is characteristic for each species. For example, six or eight were found in *Urophlyctis Rubsaameni*, nine to fifteen in *U. Alfalfae*, and fourteen to twenty-four in *U. pluriannullatus*.

On account of conflicting statements made by Saccardo and Mattiolo<sup>(22)</sup> and by Lagerheim<sup>(10)</sup> respectively with regard to the composition of the wall of the resting sporangium of *Urophlyctis Alfalfae*, I tried the usual micro-chemical tests for cellulose and cuticularised walls on the resting sporangia of the present species. In every case I obtained negative results for cellulose or cutin. Probably the wall consists principally of chitin, which appears to be the case in most of the Archimycetes.

Lagerheim (*loc. cit.*) also reported that the walls of the cavities containing the resting sporangia of *U. Alfalfae* were lignified, whereas Saccardo and Mattiolo (*loc. cit.*) found that they consisted of typical cellulose. The latter is certainly the case with the species under discussion. Scattered about, however, in the sections of the galls were isolated cells or small groups of cells, destitute of contents, which had probably previously contained the parasite, and the walls of these cells gave characteristic lignin reactions.

Incidentally, it may be mentioned that, while using cyanin

as a test for cuticularisation, I found that this stain proved very useful for showing up immature stages of the parasite in hand-cut sections of the galls. These became a deep blue colour, but the nearly or quite mature resting sporangia remained unstained.

#### DEVELOPMENT OF THE PARASITE.

The development of *Urophlyctis* has been investigated by Schroeter (24), Magnus (11-14), Maire and Tison (18), Vuillemin (31) and others, and most recently by Jones and Drechsler (9), who have furnished some beautiful figures of large portions of the thallus, skilfully dissected out of the fresh galls. The course of development is as follows. As the result of infection of an epidermal cell of the host by a zoospore of the parasite, the protoplasm of the zoospore is seen as a small rounded body within the cell attacked. The process of infection has not been actually observed by anyone, but probably takes place in exactly the same way as in certain other species of Chytridiineae in which it has been studied. In these the zoospore, after a period of swarming, becomes attached to its host, surrounds itself with a membrane, and then puts out a very fine infection-tube by means of which it pierces the external wall of its host. Through this tube the contents of the zoospore gradually pass into the epidermal cell of the host. After infection is completed, the empty wall of the zoospore is often visible on the external surface of the host.

This minute fungus cell increases in size, and develops a branched haustorial process at its apex. This cell gives rise to a small number of very fine hyphae with slightly swollen ends. With the growth in length of these hyphae, the swollen apices also increase in size, until, finally, each one comes to resemble the parent cell and is likewise terminated by an haustorium. This proceeding is repeated a few times. Each cell also normally gives rise to a resting sporangium, which arises as a small round body from the centre of the haustorium, and rapidly increases in size. It draws its nourishment from two sources, partly from the cell from which it originates, and partly directly from the host by means of a ring of haustoria, situated near the apex, to which reference has already been made.

To the cells from which the fresh hyphae and the resting sporangia arise various names have been given. Büsgen (3) termed them "Sammelzellen," A. Fischer (6) speaks of them as "Anhangszellen," Cornu employs the name "corps central," Vuillemin (31) speaks of them as "vesicules collectives," Magnus (14) designates them "antheridia," while Jones and Drechsler (9) describe them as "turbinate cells." In this paper I prefer to

make use of Büsgen's original term "Sammelzellen," which I translate as "collecting cells."

My own observations, made upon preparations obtained by teasing apart small pieces of the tissue of the fresh galls at different stages of growth, show that the general structure and development of the present fungus agree closely with other species of *Urophlyctis*, except in a few minor details. Sections, either stained or unstained, are unsatisfactory for this purpose, because the various stages in the development of the parasite are so closely crowded together within the cavities of the galls that it is impossible to observe how they are connected.

The mode of infection could not be observed, because by the time a gall has developed sufficiently to become visible infection is long past, and the few external layers of cells of the galls were always quite free from the parasites. But the presence of empty cells here and there, the walls of which reacted to stains differently from those of the other cells, made it probable that they had previously harboured stages of the parasite. The hyphae are extremely narrow, measuring about  $5\mu$  in diameter, and, as is characteristic of the genus *Urophlyctis*, these hyphae either disappear after their function is finished, or at least they can no longer be discerned. An exception to this last statement is furnished by the occurrence in the older galls of several hyphae with greatly thickened and irregular walls. These were also observed by Magnus (14), Vuillemin (31), and Jones and Drechsler (9). Magnus suggested that these thick-walled hyphae may renew their growth during the next period of vegetation. They appear, however, to be rather undergoing degeneration, and, in any case, one does not see the necessity for this mode of perennation when there is such an abundant production of resting sporangia, presumably capable of germination under suitable conditions.

The collecting cells (Figs. 7 and 10-13) have a somewhat top-shaped form, being rounded at the distal end which bears the haustorium, and tapering at the proximal end where they are attached to the hypha. They measure  $15-20\mu$  in diameter. They are at first filled with dense granular contents. When the resting sporangium arises from the haustorial process, as already described, branches of the haustorium are often visible around the base of the sporangium during the early stages of its growth (Fig. 13). The contents of the collecting cell are transferred into the growing sporangium leaving the collecting cell empty, except for a few scattered granules. The empty collecting cell is visible for a long time as a small round transparent vesicle attached to the less convex side of the resting sporangium.

Schroeter (24) was the first to observe the phenomenon described above, which he interpreted as a sexual act, and he termed

the collecting cell the antheridium, and the young resting sporangium an oosporangium. But there is insufficient evidence to support Schroeter's views.

The young resting sporangium is colourless and its external surface is smooth. It has the form of a flattened sphaeroid, and its apex is covered with the haustorial processes to which reference has previously been made (Figs. 7, 10-13). Its contents appear coarsely granular with abundant oil drops. As it increases in size the basal part becomes flatter (Figs. 9, 10), the markings upon its surface make their appearance, and the circular ridge separating the two surfaces develops. Later on, the wall increases in thickness and becomes brown, and the ornamentation of the surface is more pronounced.

A collecting cell gives rise also to fresh collecting cells, which spring from near the apex as a small number of fine hyphae with enlarged ends each terminating in an haustorial process (Figs. 9-11). In the present species the maximum number which arises from any given collecting cell is three, but in *Urophlyctis Alfalfae* and *U. pluriannullatus* the maximum number, according to Jones and Drechsler (9), is respectively four to five and seven to eight.

Often, only one or two were observed, but others may have become detached during manipulation. A collecting cell usually gives rise to others before the resting sporangium which it bears has nearly reached its full size.

In their account of the development of *Urophlyctis Alfalfae* and *U. pluriannullatus*, Jones and Drechsler (9) state that, before the collecting cell gives rise to others of a higher order, a corresponding number of peripheral lens-shaped cells are cut off from it in the neighbourhood of the apex by means of curved septa, and a fresh collecting cell arises from each of these segments. After long and careful observation, I have been unable to verify this proceeding in the present species. But I have observed several times in the teased preparations and in microtome sections that a fair-sized segment is cut off by a wall on one side of the collecting cell (Figs. 7 and 13). In one or two instances I found two segments delimited in this way on opposite sides from the upper part of a collecting cell (Figs. 12 and 19). When a segment (or two segments) was cut off in this manner, in no case did the segment or segments appear to give rise to fresh collecting cells and I am unable to explain their significance. The hyphae very rarely branch, but one instance of this was seen (Fig. 13).

## ORIGIN OF THE CAVITIES WITHIN THE GALLS.

While the parasite is growing and producing new sporangia, it is continually enlarging the cavities in the galls within which it is contained. The cell walls are the first to be broken down and dissolved, and the remains of the protoplasm and nuclei of the host cells may be seen around and among the immature collecting cells and resting sporangia of the parasite (Figs. 16 and 17). By the time all the sporangia in any cavity have reached maturity no traces remain of the host protoplasm or nuclei, since these have been used up by the parasite.

The effect of the fungus upon the nuclei of the broken-down cells is to cause a considerable enlargement of the nucleus as a whole, and of the nucleolus, which renders them very conspicuous (Figs. 16 and 17, *n.*). Bally<sup>(1)</sup> noticed the same phenomenon in *Urophlyctis Rubsaameni*, and Jones and Drechsler<sup>(9)</sup> in *U. Alfalfae*. Von Guttenberg<sup>(8)</sup> observed a similar hypertrophy of the nucleus of host cells inhabited by species of *Synchytrium*.

The wall lining the cavity is considerably thickened, but it still gives a cellulose reaction. The walls separating neighbouring cavities often show window-like openings of various shapes and sizes where the wall is in process of being broken down (Figs. 24 and 25). Magnus<sup>(12)</sup> describes and figures similar perforations in the case of *Urophlyctis pulposa*. All the cell walls of the galls stain deeply with ruthenium red, proving the abundant presence of pectic compounds.

The conclusion that I have arrived at from the study of galls at various stages of development is that their growth is brought about mainly by the activity of the cambium of the stem, aided by the cambium of the numerous vascular bundles which traverse the galls. There is never any cambial activity producing radial rows of numerous cells surrounding the cavities, such as Magnus<sup>(12, 14)</sup> describes and shows in his figures of *Urophlyctis leproides* and *U. Alfalfae*.

## TEMPORARY SPORANGIA.

No temporary sporangia have been observed in the present species. The only member of the genus for which they have been described is *Urophlyctis pulposa*, by Schroeter<sup>(24)</sup>, but Magnus<sup>(12)</sup> and Vuillemin<sup>(31)</sup> failed to find them. They were said by Schroeter to be of a huge size (up to 200  $\mu$  across), spherical or ovate in form, with bright orange contents. Beneath each sporangium is a peculiar enlarged cell into which penetrates a dense tuft of short branched hyphae constituting an haustorium (*vide* also Schroeter<sup>(25)</sup>, Fig. 70). The sporangium is surrounded



by an investment formed by the growth of the surrounding epidermal cells, suggesting, apart from the haustorium, the gall formed by certain species of the genus *Synchytrium*. The zoospores which escape by a pore at the apex, are described as spherical in form and comparatively large, with one cilium and a refringent oil-globule. This description fits exactly the zoospores also of *Synchytrium*.

#### GERMINATION OF RESTING SPORANGIUM.

Attempts made to germinate the resting sporangia so far have not been successful. They have been sown in water and in various culture solutions, kept at various temperatures. They have been subjected to artificial freezing. The galls were observed to have been occasionally gnawed by slugs, and it was thought that possibly slugs might be instrumental not only in the dissemination, but also in bringing about the germination of the sporangia. Elliott<sup>(4)</sup> has made some observations on the mycophagous propensities of slugs. A few specimens of the common field slug (*Agriolimax agrestis*) were first subjected to a short period of starvation to empty the alimentary canal, after which they readily devoured the galls. The faeces consisted almost entirely of resting sporangia, but attempts made as before to germinate these gave negative results. Bally<sup>(1)</sup> states that he has observed the germination of the resting sporangia of *Urophlyctis Rubsaameni*, which took place readily after they had lain for some time in water. An active movement of the zoospores was first visible in the interior of the sporangium. Irregular clefts then appeared in the exospore, followed by the rupture of the exospore first, and then the endospore. The escaping zoospores had a diameter of  $1-3\mu$  and were provided with a single cilium. They performed rotating movements similar to those of *Synchytrium endobioticum*, and exhibited in their cytoplasm two points coloured black by the vapour of osmic acid. The resting sporangium never became completely emptied, but a certain amount of protoplasm with oil drops remained behind.

In the autumn of 1922, plants of bird's-foot trefoil bearing galls were planted in a flower-pot, and left outside during the winter to ascertain whether, after a lengthy exposure to atmospheric influences, the resting sporangia could be induced to germinate. The flower-pot, however, unfortunately met with an accident. A similar experiment was again tried during the winter of 1924-5, but no germination could be obtained when the resting sporangia were sown in water and nutrient solutions during the following spring and summer.

## CYTOLOGY.

In the youngest stage found of the collecting cell a single nucleus only was present, which it probably received from a collecting cell of a lower order, according to the observations of Jones and Drechsler (9). As the collecting cell grows in size the nuclei within it increase in number. Figs. 8, 26, and Figs. 16 and 17, *c.c.* represent collecting cells in different stages of development. Within each nucleus of the collecting cell is seen a deeply staining, round or oblong nucleolus or karyosome, usually occupying a more or less central position. Surrounding this is a clear area, the nuclear vacuole, which appears to be delimited externally by a nuclear membrane. The fine hyphae which join together the collecting cells contain no nuclei. With reference to the mode of multiplication of the nuclei, occasionally figures were seen which suggest an indirect division with the formation of a spindle (Fig. 15), but on account of the very small size of the nuclei in the collecting cells nothing definite can be stated. Bally (1), Fron and Lasnier (7), Maire and Tison (18), and Jones and Drechsler (9), all agree in describing the nuclear divisions as amitotic. The surrounding cytoplasm is dense and finely granular, and stains somewhat deeply with nuclear stains. It is bounded externally by a very delicate cell wall.

When the resting sporangium arises as an outgrowth from the centre of the haustorium, at the apex of the collecting cell, the nuclei and cytoplasm contained in the latter are gradually transferred into the young resting sporangium. Fig. 22 represents a stage in the process, in which a nucleus is seen squeezing its way through the narrow connection between the resting sporangium (*r.s.*) and the collecting cell (*c.c.*). Finally, the collecting cell becomes entirely emptied of its contents.

Very soon after the nuclei have passed into the young developing resting sporangium, a number of them undergo a considerable increase in size, which involves not only the nucleus as a whole but also the nucleolus. The nucleolus is then found in contact with the nuclear membrane, and a distinct chromatin network is seen occupying the nuclear vacuole. The nuclear membrane becomes thickened and very clearly defined (Figs. 16, *r.s.*, 18, 19, and 21). Either the enlargement of the nuclei takes place very rapidly, or the process of transference of the contents of the collecting cell is a slow one, because in Fig. 22 one of the nuclei in the resting sporangium is seen to have become considerably enlarged whilst the collecting cell has lost only a part of its contents. These nuclei attain a diameter of 5 to 6  $\mu$  and their nucleoli measure 2 to 2.5  $\mu$  across. Concurrently with the enlargement of the nuclei, spherical grains of chromatin of various

sizes, staining a deep black with iron haematoxylin, make their appearance scattered throughout the cytoplasm of the developing resting sporangium (Figs. 16, *r.s.*, 18, 19 and 21). The appearance of granules of chromatin in the general cytoplasm, as well as the enlargement of some of the nuclei and nucleoli, are likewise reported as occurring in *Urophlyctis hemisphaerica* and *U. Alfalfae* by Maire and Tison<sup>(18)</sup> and Jones and Drechsler<sup>(9)</sup> respectively. These observers state that the origin of the chromatin in both of the above species is due to the swelling and final disintegration of some of the nuclei. I have been unable to confirm this observation in the case of the present species, but am of opinion that the nuclei diminish in number while the resting sporangium is increasing in size. At the same time the general cytoplasm becomes less dense and shows a distinctly reticulate appearance. In the mature sporangium the granules of chromatin collect in the centre to form a large deeply staining mass of irregular form (Figs. 17, *r.s.*, 20 and 23). The nuclei are found situated outside this mass, and are often difficult to observe.

In the meanwhile, the resting sporangium loses its almost spherical form, and becomes transversely ellipsoidal and its wall becomes thickened and acquires the characteristic ornamentation; afterwards, the future base undergoes a still further flattening. The wall does not increase uniformly in thickness throughout, but at first the thickening is most pronounced at the base and apex (Fig. 21), the circumference remaining thin for a time and thus allowing for further extension.

In the mature sporangium (Fig. 20) the wall is thickest in the centre of the base and apex, and somewhat thinner around the circumference immediately above the prominent ridge where it acquires its greatest thickness. Two distinct layers are visible in a section through the wall, the endospore and the exospore (Fig. 20, *en.* and *ex.*).

#### GENERAL CONSIDERATIONS, AND SYSTEMATIC POSITION OF THE PARASITE.

The fungus evidently belongs to the family Cladochytriaceae A. Fischer (*sensu restricto*), which in a critical survey of the entire group of the Chytridiineae, not yet published, I have characterised as follows:

"Mycelium well-developed, consisting of simple or branched hyphae, which bear several either terminal or intercalary sporangia. Sporangia of two kinds, temporary, or resting, both formed asexually. Hyphae usually disappearing when the sporangia are mature. Mostly parasitic within the tissues of seed-plants."

Four genera are included in this family, viz. *Cladochytrium*, *Physoderma*, *Urophlyctis*, and *Nowakowskiella*. The characters by which these genera are distinguished from one another have not been very clearly defined in the past with the result that certain species have been shifted about from one genus to another by different authorities. The life history, also, of most of the species is very imperfectly known.

A. Fischer (6) placed all the species then known to him in the single genus *Cladochytrium* which comprised the three subgenera *Cladosporangium*, *Urophlyctis*, and *Physoderma*. There is much to be said for his point of view, because all the members of this family appear to be closely related. However, he based the diagnosis of his three subgenera principally upon the presence or absence, and the characters of the zoosporangium or temporary sporangium. Since the latter has been reported for only a single species of *Urophlyctis*, and the isolated observation has not been confirmed, it does not seem to me to afford satisfactory or safe characters to be used for the purposes of classification.

Schroeter (25), on the other hand, claimed the existence of four separate genera, but he placed the genus *Urophlyctis*, founded by himself, in a separate family, Oochytriaceae, on account of a supposed sexual process in the formation of the resting sporangium, to which reference has already been made. Even if there were sufficient evidence to support Schroeter's assumption, little can be said in defence of this arrangement, because it removes *Urophlyctis* from the other genera, to which it is evidently closely related, and places it in a heterogeneous group containing the genera *Diplophysa* (= *Olpidiopsis*), *Polyphagus*, and *Zygochytrium*, with none of which does it exhibit near resemblances in its other characters. Thus, the above is a purely artificial classification.

Magnus (13) shared Schroeter's views on the occurrence of a sexual process in *Urophlyctis*. He published detailed accounts of several new species of *Urophlyctis* (11-15) and did much to increase our knowledge of the genus. As the result of his investigations, he came to the conclusion that the hemispherical form of the resting sporangium, to the lower more or less flattened surface of which is attached a colourless empty cell, furnished a character by which the genus *Urophlyctis* might be distinguished from the closely allied genus *Physoderma*. In the latter the resting sporangium is uniformly oval, and the collecting cell is attached at any point. Furthermore, he pointed out that *Urophlyctis* causes a considerable enlargement of the host cells, the walls of which become swollen and mucilaginous, and are eventually partially absorbed. But this character does

not apply to all the species, because in *Urophlyctis Urgineae* (Pat. & Trab.) Maire, if indeed this is a *Urophlyctis*, the walls of the host cells appear to be not at all affected.

The present fungus, then, should be included in the genus *Urophlyctis*, as defined by Magnus, which contains the following species:

1. *U. major* Schroet. Krypt. Fl. Schles. III, i, Pilze, p. 197, 1886. In leaves of *Rumex* sp.
2. *U. pulposa* Schroet. Ibid. In leaves, stems and perigonia of *Chenopodium* spp. and *Atriplex* spp.
3. *U. Alfalfae* (Lagerheim) Magn. in Ber. Deutsch. Bot. Ges. xx (1902), 291-296. On *Medicago* spp.
4. *U. Rubsaameni* Mag. Ibid. xix (1901), (145)-(153). In roots of *Rumex scutatus*.
5. *U. Trifolii* (Pass.) Magn. in Centralbl. f. Bakt. ix (2) (1902), 895-897. On leaves, petioles and peduncles of *Trifolium* spp.
6. *U. hemisphaerica* (Speg.) Syd. in Ann. Mycol. i (1903), 517-518. On leaves of several species of Umbelliferae.
7. *U. punctiformis* Spegazzini in Ann. Mus. Nac. Buenos Aires, xviii (1909), 284. In leaves of *Hypochaeris variabilis* and *Picrosia longifolia*.
8. *U. Magnusiana* Neger in Ann. Mycol. iv (1906), 280-282. In stems, leaves and calyces of *Euphrasia Odontites*.
9. *U. Urgineae* (Pat. & Trab.) Maire. Bull. Soc. Bot. France, LIII (1906) clxxiv. In leaves of *Urginea Scilla* and *U. maritima*.
10. *U. pluriannulatus* (Berk. and Curt.) Farlow in Rhodora, x (1908), 12-13. In leaves, petioles and stems of *Sanicula* sp. and *Ligusticum apitifolium*.
11. *U. Asphodeli* (Debray) Maire in Bull. Soc. Bot. France, LIII (1906), clxxiii. In leaves of *Asphodelus microcarpus* and other species.

If we examine the hosts in or upon which the above species occur, we shall observe that the genus *Urophlyctis* exhibits a somewhat closely specialised parasitism, since each species is confined to either a single genus of flowering plants, or, at the most, to a few genera belonging to the same Natural Order. Furthermore, each species attacks a definite part or parts of the host. Hence, it is unlikely that the present species, parasitic upon *Lotus corniculatus*, would attack other than Leguminous plants.

Of the species of *Urophlyctis* already described two only are found upon plants belonging to the Leguminosae, viz. *U. Alfalfae* on Lucerne and another species of the genus *Medicago*, and *U. Trifolii* on certain other species of *Trifolium*. *U. Trifolii* may be dismissed from our consideration, because it attacks only the subaerial parts of the host, i.e. the leaves, petioles and peduncles, resulting in the production of minute hemispherical warts. Moreover, the wall of the resting sporangium is described as smooth, as it is also in *U. Alfalfae*.

Further characters in which *U. Alfalfae* differs from the species on *Lotus corniculatus*, are found in the large irregular coral-like galls produced by the former, and the much smaller, smooth, rounded galls caused by the latter. Differences in the structure of these two kinds of galls have already been pointed out. Other differences may be noted in the resting sporangia.

The number of pits in the wall through which the sporangia emerge were found by Jones and Drechsler<sup>(9)</sup> to be nine to fifteen for *U. Alfalfae*, and they are described as forming a circle which occupies a position a little above the equator. The pits number five to nine in my species, and they form an irregular circle situated much nearer to the apex.

Lastly, the present species differs from all those previously described in the characteristic ornamentation of the surface of the resting sporangium, as well as in the distinct angular ridge separating the more convex from the flatter surface. In the accounts or figures of all the other species of *Urophlyctis*, the external surface is described or represented as smooth, the wall as of even thickness throughout, and the zone between the upper and under surfaces as rounded.

From the above considerations, I believe that I am justified in regarding the *Urophlyctis* producing galls on *Lotus corniculatus* as a new species, to which I propose to give the name of *Urophlyctis Potteri*, after Dr M. C. Potter, Professor of Botany at Armstrong College, who first drew my attention to these galls. It may also be regarded as a slight token of my affection and esteem for Professor Potter, who has recently retired after thirty-five years of arduous and distinguished service as Professor of Botany at Armstrong College.

### *Urophlyctis Potteri* sp. nov.

In basi caulium erectorum aut in caulibus hypogaeis *Loti* corniculati generans excrescentias globulosas usque ad 1 cm. diam., excrescentias plenas sporangiis perdurantibus in variis lacunis, sporangiis hemisphaericis sub-brunneis exornatis supra inordinatis radiantibus sulcis, infra convexiusculis granulatisque, 40–50  $\mu$  diam. 25–30  $\mu$  altis.

*Hab.* In basi caulium *Loti* corniculati prope Ponteland, Northumberland, England.

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## DESCRIPTION OF PLATES XI—XIV.

Fig. 1. Photograph of galls on plants of *Lotus corniculatus*. Nat. size.

Fig. 2. Photomicrograph of section of gall.  $\times 50$ .

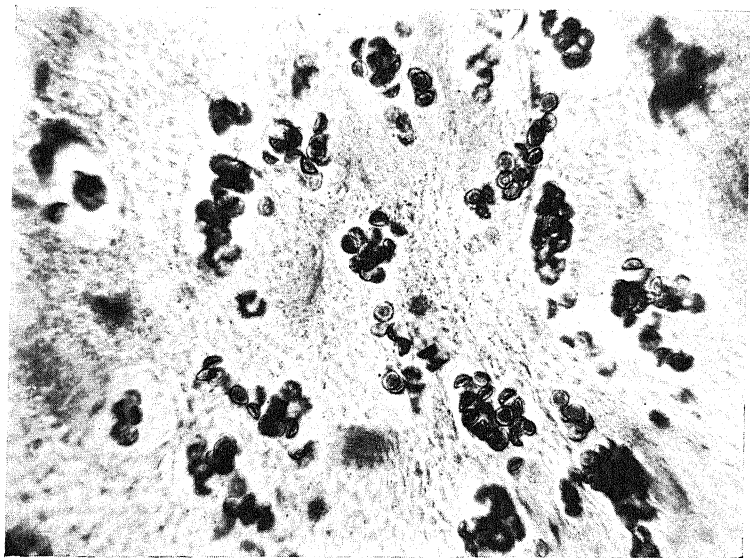
Fig. 3. Photomicrograph of section of gall.  $\times 150$ .

Fig. 4. Photomicrograph of a cavity in a gall containing resting sporangia.  $\times 350$ .





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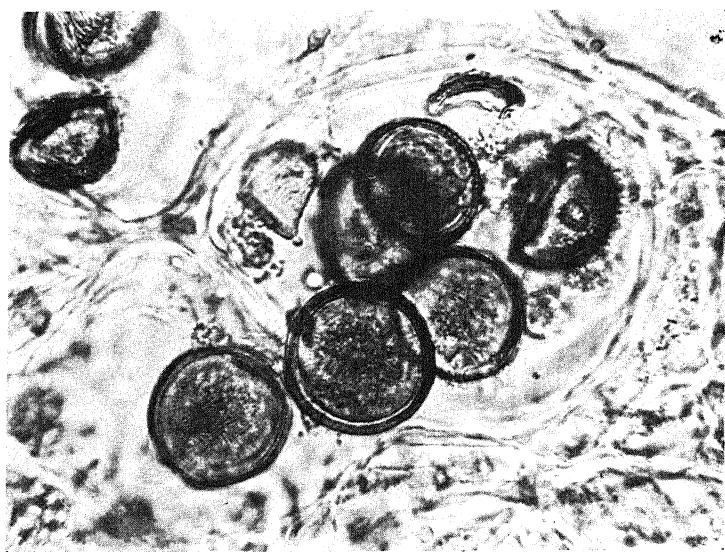
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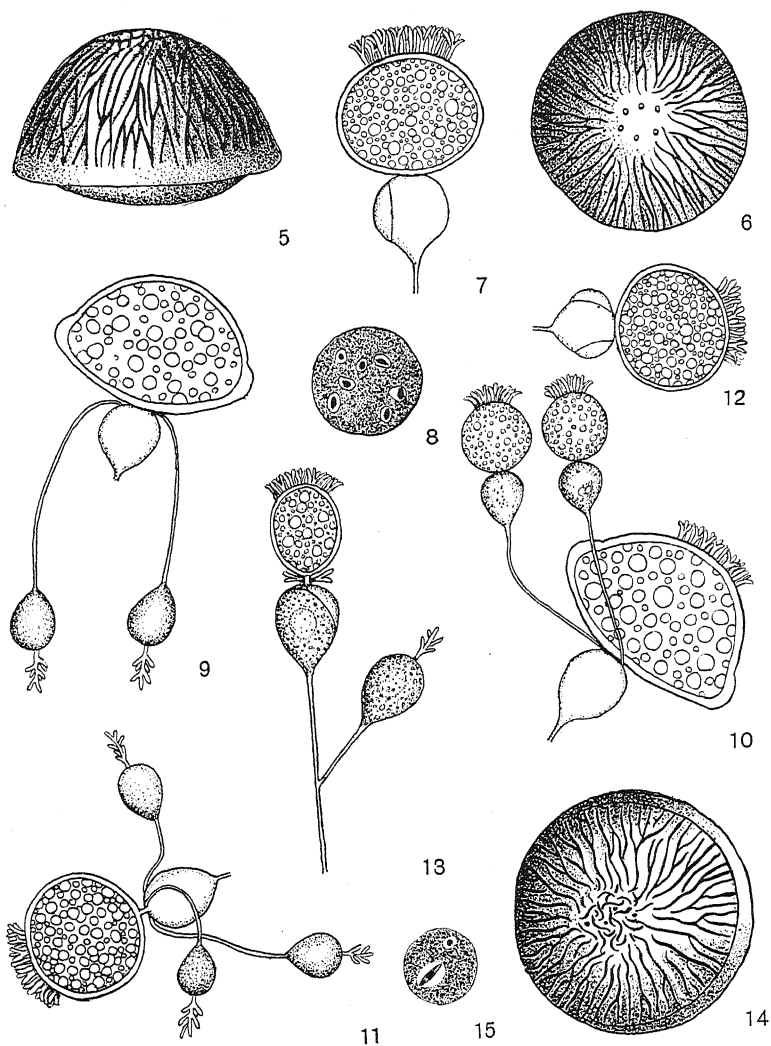
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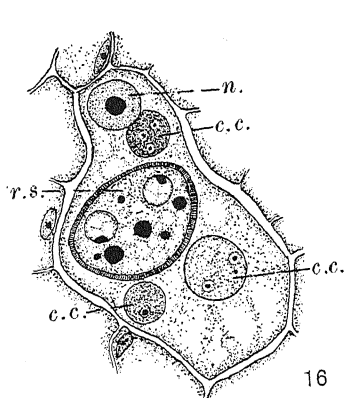
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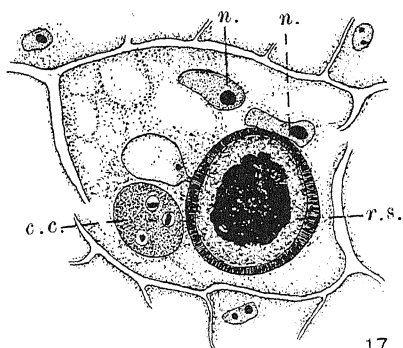


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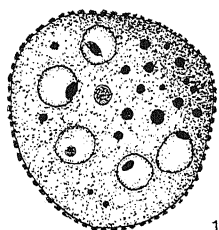




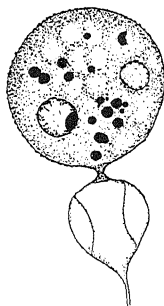
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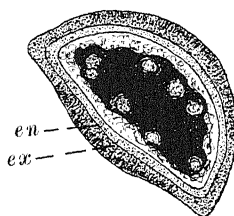
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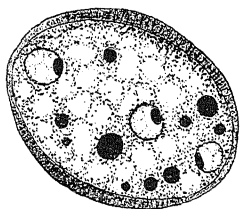
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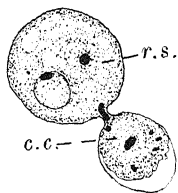
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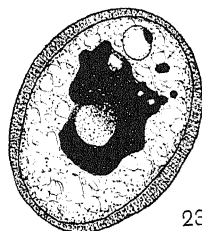
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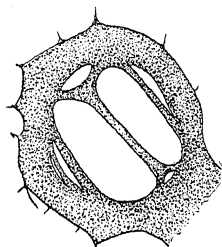
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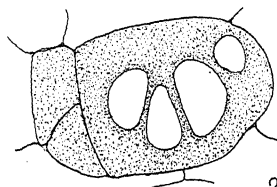
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- Fig. 5. Mature resting sporangium, seen from the side.  $\times 700$ .  
 Fig. 6. Immature resting sporangium, dorsal view.  $\times 700$ .  
 Fig. 7. Young resting sporangium with empty collecting cell.  $\times 700$ .  
 Fig. 8. Collecting cell in transverse section.  $\times 700$ .  
 Figs. 9-13. Immature resting sporangia in various stages of development, showing origin of new collecting cells and sporangia.  $\times 700$ .  
 Fig. 14. Mature resting sporangium, from above.  $\times 700$ .  
 Fig. 15. Early stage of collecting cell, containing a nucleus apparently undergoing karyokinesis.  $\times 1000$ .  
 Figs. 16 and 17. Sections of cavities in galls, showing collecting cells (*c.c.*) and resting sporangia (*r.s.*) in various stages of development. *n.*, *n.* are enlarged nuclei of host-cells.  $\times 500$ .  
 Figs. 18, 19 and 21. Sections of resting sporangia, showing stages in development.  $\times 800$ .  
 Fig. 20. Median vertical section of mature resting sporangium, containing large central mass of chromatin. *en.*, endospore; *ex.*, exospore.  $\times 700$ .  
 Fig. 22. Young resting sporangium (*r.s.*) receiving contents of collecting cell (*c.c.*). A nucleus is seen in the narrow passage connecting the two.  $\times 800$ .  
 Fig. 23. Maturing resting sporangium with large central mass of chromatin.  $\times 800$ .  
 Figs. 24 and 25. Perforations in cell-walls between adjoining cavities.  $\times 800$ .  
 Fig. 26. Section of immature collecting cell.  $\times 800$ .

## A NOTE ON BOTRYODIPLODIA SP. ON CHOISYA TERNATA IN ENGLAND

(With 4 Text-figs.)

By R. C. Woodward,

*School of Rural Economy, Oxford.*

A PYCNIDIAL fungus causing a dying back of the extremities of twigs of *Choisya ternata* was investigated in the spring of 1923. The organism isolated from dead branches of this shrub kindly sent by Mr W. J. Dowson of Wisley and also from specimens in the Cambridge Botanic Gardens proved to be a species of *Botryodiplodia*, a genus rare in temperate regions but widespread both as a saprophyte and as a parasite in the tropics.

The fungus, of which a single spore isolation was made, produced a good vegetative growth on potato agar similar in character to that of *Botryodiplodia Theobromae* Pat. Sterilised twigs of *Choisya ternata* in culture tubes served as a very satisfactory medium; the fungus penetrated all the tissues and eventually formed pycnidia in the bark. After some time soft cushion-like masses of vegetative growth of a sooty appearance were formed. The brown, thick-walled, uniseptate spores germinated rapidly and vigorously in water; they retained their vitality over long periods and resisted desiccation. Spores, in a sterilised twig-culture, germinated freely when immersed in water after remaining three years in a dry condition.



A number of inoculations were carried out on a healthy shrub of *Choisya ternata* in the Botanic Gardens, Cambridge. A pure culture of mycelium was used as the inoculum; inoculations were made with a sterile scalpel, and uninoculated, but otherwise similarly treated branches, served as controls; all the controls remained uninfected. Ten out of twelve inoculations proved successful.

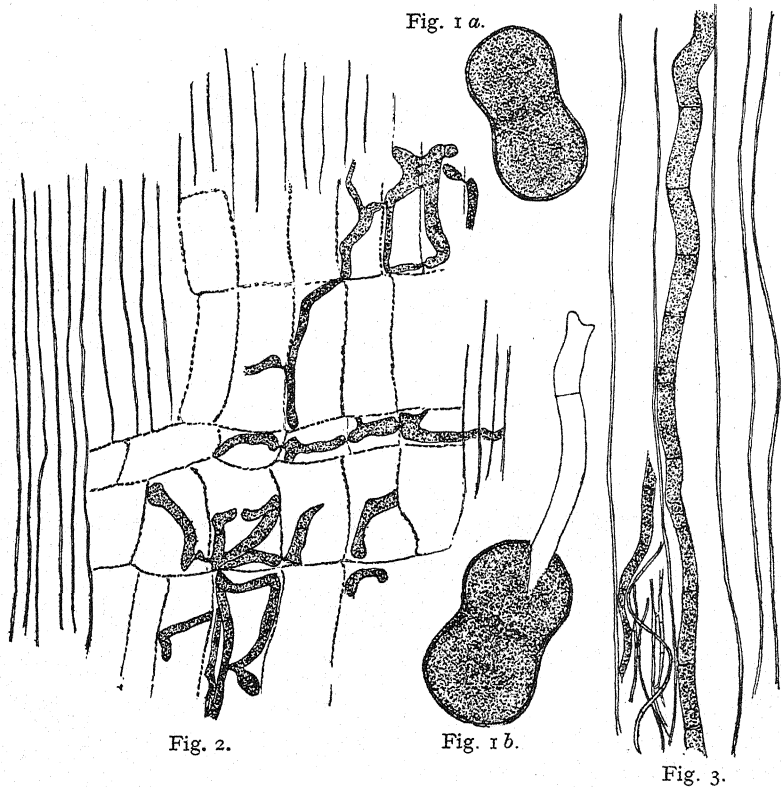


Fig. 1 a. Typical uniseptate spore.

Fig. 1 b. Germinating spore with hyaline germ-tube.

Fig. 2. Characteristic appearance of mycelium in medullary ray. Note the penetration of cell-walls by way of pits.

Fig. 3. Large, dark-brown hyphae in the vessels accompanied by smaller hyaline hyphae.

The fungus is remarkable for its rapidity of attack; inoculations made in May on living healthy branches killed them outright within a month.

The dead branches presented a great contrast to the healthy ones owing to the brown withered leaves which persisted on

them. In a successful inoculation a darkened area usually occurred near the callus which had formed over the inoculation wound. On splitting the branch longitudinally the woody tissue in the vicinity of the wound was seen to be discoloured; the pith was brown, the discoloration extending further in this region than in the wood. The penetration of cell walls takes place by way of the pits. The hyphae frequently anastomose. The pith and wood are darkened by the more mature hyphae, which are dark brown in colour. The darkened tissues, however, do not indicate the full area of invasion; thin, hyaline hyphae may be seen extending further in the vessels and medullary rays. Whereas no staining is required to show the distribution of the older hyphae, delicate staining is necessary to differentiate the very fine hyaline hyphae which initiate and extend the invasion of the tissues. The death of the infected branch is probably caused by the cutting off of the water supply by hyphae in the vessels. Fructifications were formed in the dead bark; they were frequently close together, forming a continuous slightly projecting mass of minute pinnacles.

In the absence of host plants upon which inoculations might be carried out to establish the species the specific name *Botryodiplodia Theobromae* Pat. may be only provisionally accepted.

The fungus has received a large number of different names, among which are *Macrophoma vestita*, *Diplodia cacaicola*, *Lasiodiplodia Theobromae*, and *Diplodia rapax*. Petch<sup>(1)</sup> gives the following host plants: cacao, sugar cane, *Albizia Moluccana*, papaw, mango, castilloa, *Hevea brasiliensis*, dadap, *Ficus elastica*, tea, and coconut. It has recently been reported as causing a gumming degeneration of tissues of the tobacco plant in Brazil<sup>(2)</sup> and as an apple root disease in Kenya<sup>(3)</sup>.

My thanks are due to Mr F. T. Brooks, who suggested an investigation of this fungus and who pointed out its similarity to *Botryodiplodia Theobromae* as seen in the tropics. This work was done in the Cambridge University Botany School.

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## ON TWO SPECIES OF *TOLYPOSPORIUM* WORONIN RECORDED ON CULTIVATED SORGHUM

By E. W. Mason, M.A., M.Sc.,  
Imperial Bureau of Mycology.

### I. *Tolyposporium Ehrenbergii* (Kühn) Pat. = *Tolyposporium filiferum* Busse.

LAST year (1925) Zaprometoff published a book on the diseases of plants in Middle Asia, and in it gave an account of *Sorosporium Ehrenbergii* Kühn on sorghum (*Andropogon Sorghum*). On request, he kindly supplied specimens of the fungus on *Andropogon Sorghum contractus* Kcke., collected by A. Pospeloff in the Bokhara region in August, 1925. They were recognised, on receipt, as the same smut which is recorded in Africa and India as *Tolyposporium filiferum* Busse. As Kühn's name antedates Busse's by many years, it became necessary to determine whether Zaprometoff's determination could be substantiated.

The history of *Sorosporium Ehrenbergii* is as follows. Schweinfurth collected specimens at Cairo, Egypt, in 1876, and forwarded them to Thümen, who issued them in his *Mycotheca Universalis*, No. 725, as *Ustilago Reiliana* Kühn f. *Sorghicernui* on *Sorghum cernuum* Willd. [*Andropogon Sorghum Brot.*]. Kühn examined a specimen and finding that it was not his species, and not even congeneric with it, named it *Sorosporium Ehrenbergii*.

Thümen's *Mycotheca Universalis*, No. 725 in Herb. Kew, is identical with Zaprometoff's material, and there can be no doubt that it is Kühn's species of which a full account is given in *Hedwigia*, xvii (1878), pp. 12-14. His species is differentiated from the other smuts on sorghum by the aggregation of its spores into spore balls, as in *Sorosporium bullatum* Schroet. [= *Tolyposporium bullatum* Schroet. 1887] on *Echinochloa crus-galli*. Unlike the latter species, however, the individual spore sacs are remarkably long, 8 to 13 mm. long and 3 to 5 mm. broad. The usual diameter of the spores is 12.4  $\mu$ .

As Thümen's No. 725 in Herb. Kew shows just these characters, it must be taken that it is genuine *Sorosporium Ehrenbergii* Kühn, and that Zaprometoff's determination is correct. Further, Busse's description and figures of his *T. filiferum* leave no doubt that he was dealing with the same fungus. Sydow's *Fungi Exotici Exsiccati*, No. 351, in Herb. Imp. Bur.

Myc., issued as *Tolyposporium filiferum* collected by W. McRae, Government Farm, Coimbatore, Madras Presidency, India, is identical with the Egyptian and Middle Asian material. The specific name *filiferum* must, therefore, be abandoned. On the other hand, the presence of the persistent spore-balls places the fungus in the genus *Tolyposporium*. The first time I have found it definitely listed in this genus is in 1903 by Patouillard. The correct citation therefore appears to be

*Tolyposporium Ehrenbergii* (Kühn) Pat. in Bull. Soc. Mycol. XIX (1903), p. 254. Synon. *Sorosporium Ehrenbergii* Kühn in Die Brandenformen der Sorghumarten. Ein Beitrag zur Geographie der Pflanzenkrankheiten. Mitt. Ver. Erdkunde Halle (1887), p. 87 (not seen); and Hedwigia, XVII (1888), p. 13. *Tolyposporium filiferum* Busse in Untersuchungen über die Krankheiten der Sorghum-hirse. Arb. K. Biol. Abt. für Land-Forstwirtsch. IV (1904), 4, p. 384.

The fungus has been recorded as *T. filiferum* in Tanganyika territory (formerly German East Africa) by Busse (*loc. cit.*); in British India by Sydow and Butler (1906), by Butler (1918) and by Kulkarni (1918); in Egypt, the type locality of *S. Ehrenbergii*, by Briton-Jones (1922), who states that it is there the commonest smut of sorghum. In 1921, Thomas baldly recorded the "long smut" of sorghum for Mesopotamia; long smut is a popular name for this fungus, but no description is given. Under the specific name of *Ehrenbergii*, it is recorded from Egypt by Kühn (*loc. cit.*), from Tunis by Patouillard (*loc. cit.*) and now from Central Asia by Zaprometoff (1925).

The smut can easily be recognised by the following characters:

- (1) the long spore sacs, each confined to one spikelet of the host;
- (2) the presence of a number (up to ten) of brown threads in each spore sac, in place of a central columella;
- (3) the aggregation of the spores into spore balls, which do not fall to pieces under the coverslip;
- (4) the angled spores with a mean diameter of about 12  $\mu$ .

It is satisfactory to note that the fourth and last smut of sorghum known to Kühn has been recognised again, in view of Reed's (1925) statement that while Kühn's description corresponds closely with *Sorosporium Reilianum*, the specimen of Thümen's *Mycotheca Universalis*, No. 725, at the New York Botanical Gardens, cannot be distinguished from *Sphacelotheca Sorghi*. Kühn's real species is clearly distinct from either of these.

## II. *Tolyposporium Volkensii* Henn.

This species on cultivated *Sorghum* sp. was founded by Hennings in Engler, *Die Pflanzenwelt Ost-Afrikas und der Nachbargebiete*, Theil C, p. 49 (1895), on a collection made by

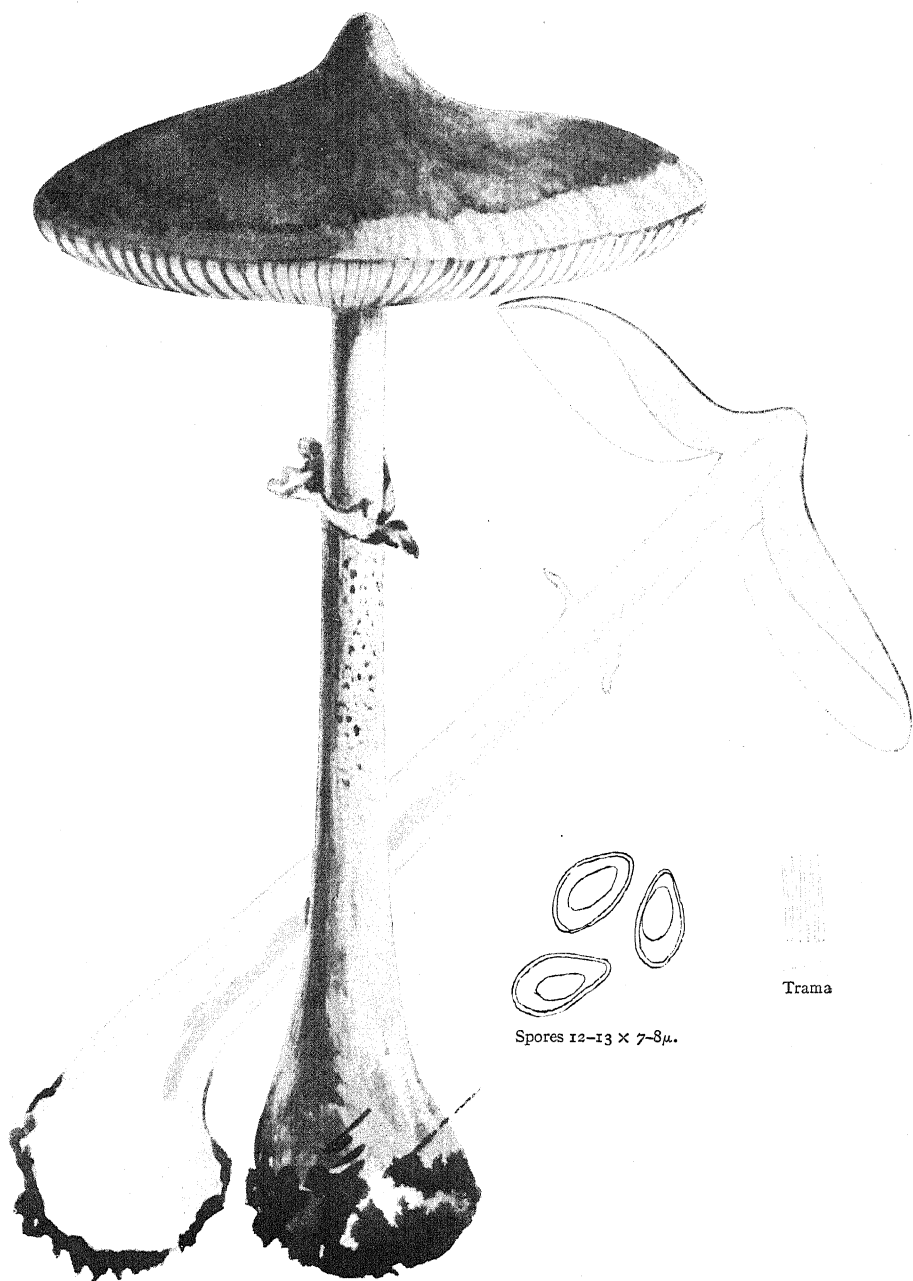
Volkens at Kilimandscharo, East Africa. The species does not appear to have been recognised again, and there has been some speculation among tropical mycologists as to the identity of this species. A collection in Herb. Kew, labelled *Tolyposporium Volkensii* Henn., Flora des Kilimandscharo No. 296, Marangu, May 1893, leg. G. Volkens, had been referred there to *Cerebella Andropogonis* Ces.

As the authenticity of this material was doubtful, Hennings's type specimen was obtained through the courtesy of Professor Diels, Director of the Botanical Museum, Friedrich-Wilhelm's Universität, Berlin-Dahlem, and the material at Kew proved to be genuine *Tolyposporium Volkensii* Henn. It is also identical with a collection received from J. McDonald, Kenya, and which was referred at this Bureau to *Cerebella Sorghi-vulgaris* Subram. (*Journ. and Proc. Asiatic Soc. of Bengal* (N.S.), xvii (1921), 206). Subramaniam has kindly confirmed this latter determination.

The species is a good *Cerebella*, and easily to be distinguished from a smut. Under a lens, each black stroma can be seen to be folded like the convolutions on the surface of the brain, and a section shows that the stroma is covered with a palisade of conidiophores, each of which carries at its apex a conidium with septa in three planes. The correct specific names to apply to the *Cerebella* spp. on cultivated sorghums, if there are more than one, is not yet quite clear. Mr McDonald is now studying them in culture, and it is hoped that this will lead to more definite conclusions. The present note is only intended to record that *Tolyposporium Volkensii* Henn. (1895) recorded on sorghum from East Africa is not a smut but a species of *Cerebella*, and that it is identical with *Cerebella Sorghi-vulgaris* Subram. (1921), recorded on the same host from India and the Philippines.

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*Lepiota gracilentata* (Krombh.) Fr.



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219. McFarland, Dr Frank T., Department of Botany, University of Kentucky, Lexington, Ky., U.S.A. (1924.)  
220. McLean, Professor R. C., M.A., D.Sc., F.L.S., Botany School, University College, Cardiff. (1922.)  
221. McLennan, Dr Ethel I., Botanical Department, Melbourne University, Carlton, Victoria, Australia. (1926.)  
222. Nagpur, The Mycologist to the Government, C.P., India. (1924.)  
223. Nattrass, Mr R. M., B.Sc. (Agric.), Research Station, Long Ashton, Bristol. (1925.)  
224. Nebraska, The Library, University of, Lincoln, Nebr., U.S.A. (1924.)  
225. Nederlandsche Mycologische Vereeniging, c/o H. A. A. van der Lek, Zoomweg 10, Wageningen, Holland. (1920.)  
226. Newcastle-upon-Tyne, Literary and Philosophical Society, c/o Mr H. Richardson, Librarian. (1902.)  
227. Newton, Mr W. C. F., B.Sc., John Innes Horticultural Institute, Mostyn Road, Merton, Surrey. (1922.)  
228. New York Botanical Garden, Bronx Park, New York, U.S.A. (1904.)

229. Nicholson, Mr W. E., F.L.S., 50, St Anne's Crescent, Lewes.  
(1913.)
230. Noel, Miss E. F., F.L.S., 37, Moscow Court, Queen's Road,  
London, W. 2. (1913.)
231. North Carolina, Library, University of, Chapel Hill, North  
Carolina, U.S.A. (1920.)
232. Nursery and Market Garden Industries' Development Society,  
Ltd., Experimental and Research Station, Cheshunt, Herts.  
(1922.)
233. O'Connor, Mr P., B.Sc., A.R.C.Sc.I., College of Science, Upper  
Merriem Street, Dublin. (1925.)
234. Ogilvie, Mr L., M.A., M.Sc., Department of Agriculture, Agri-  
cultural Station, Paget East, Bermuda. (1922.)
235. Oke, Mr Alfred William, B.A., F.G.S., F.L.S., 32, Denmark  
Road, Hove, Sussex. (1908.)
236. Oldham, Mr C. H., Ivy Dene, Chandler's Ford, Southampton.  
(1923.)
237. Ontario Agricultural College, Library, Guelph, Ontario, Canada.  
(1920.)
238. Osborn, Professor T. G. B., M.Sc., Adelaide University, Adelaide,  
South Australia. (1910.)
239. Ottawa, Ontario, Canada, The Library, Geological Survey.  
(1926.)
240. Overeem, Dr C. van, Buitenzorg, Java. (1920.)
241. Page, Miss W. M., B.Sc., 19, Ledam Buildings, Bourne Estate,  
Holborn, London, E.C. 1. (1921.)
242. Pan, Mr T. C., M.B., Ch.B., Avondale, Lenzie, Glasgow. (1925.)
243. Parke Davis & Co., Librarian, Research Department, Detroit,  
Michigan, U.S.A. (1920.)
244. Paul, The Very Rev. David, D.D., LL.D., 53, Fountainhall  
Road, Edinburgh. (1899.)
245. Paulson, Mr Robert, F.L.S., F.R.M.S., Glenroy, Cecil Park,  
Pinner, Middlesex. (1918.)
246. Peacock, Dr H. G., The Lawn, Torquay. (1896.)
247. Pearson, Mr Arthur A., F.L.S., 59, Southwark Street, London,  
S.E. 1. (1911.)
248. Peklo, Dr Jaroslav, Professor of Applied Botany, Bohemian  
Technical University, Charles Square, Prague II, Czecho-  
Slovakia. (1924.)
249. Perthshire Society of Natural Science, c/o Mr James Winter (Hon.  
Treasurer), 35, George Street, Perth. (1919.)
250. Petch, Mr T., B.A., B.Sc., Tea Research Institute, Nuwara  
Eliya, Ceylon. (1911.)
251. Pethybridge, Mr G. H., Ph.D., B.Sc., Ministry of Agriculture,  
Pathological Laboratory, Milton Road, Harpenden, Herts.  
(1919.)

252. Philadelphia, The Academy of Natural Sciences of Philadelphia, Logan Square, Phila., U.S.A. (1925.)
253. Phillips, Dr H. H., 6, St John's Road, Penge, London, S.E. 10. (1923.)
254. Phillips, Mr F. J., Research Officer, Forest Research Station, Deepwells, Knysna, South Africa. (1921.)
255. Ping, Mr A. Wentworth, M.A., "St Olave's," Clifton, York. (1926.)
256. Plowright, Mr C. T. M., B.A., M.B., King Street, King's Lynn. (1901.)
257. Potter, Rev. M. C., Sc.D., M.A., F.L.S., Corley Cottage, York Avenue, New Milton, Hants. (1896.)
258. Povah, Professor A. H., 9, Fisk Hall, Northwestern University, Evanston, Ill., U.S.A. (1924.)
259. Preston, Mr N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop. (1920.)
260. Pretoria, South Africa, The Chief, Division of Botany (91403), Department of Agriculture. (1922.)
261. Priestley, Professor J. H., D.S.O., B.Sc., F.L.S., Botanical Department, The University, Leeds. (1912.)
262. Priestley, Mrs Marion E., 2, Balmoral Terrace, Shaw Lane, Headingley, Leeds. (1919.)
263. Pusa, Imperial Mycologist, Imperial Agricultural Research Institute, Pusa, Bihar, India. (1921.)
264. Ramsbottom, Mr J., O.B.E., M.A., F.L.S., British Museum, Cromwell Road, South Kensington, London, S.W. 7. (1910.)
265. Rayner, Mr J. F., Swaythling, Southampton. (1902.)
266. Rayner, Miss M. Cheveley, D.Sc., Bedford College for Women, Regent's Park, London, N.W. 1. (1921.)
267. Rea, Mrs E. A., 6, Barbourne Terrace, Worcester. (1896.)
268. Rea, Miss M. W., M.Sc., Salem House, Sydenham, Belfast, Ireland. (1920.)
269. Rea, Miss Violet, 6, Barbourne Terrace, Worcester. (1921.)
270. Reichert, Dr Israel, Plant Pathologist, Palestine Zionist Executive Agricultural Experiment Station, Tel-Aviv, Palestine. (1924.)
271. Rhind, Mr Donald, B.Sc., Mycologist, Department of Agriculture, Agricultural College, Mandalay, Burma. (1922.)
272. Rhodes, Miss Mabel, Lister Institute, Chelsea Gardens, London, S.W. 1. (1921.)
273. Richards, Mr R. M., M.B.E., A.R.C.S., F.L.S., The Laboratory, Caledonia Estate, Province Wellesley, Straits Settlements. (1915.)
274. Roberts, Mrs A. W. Rymer, The End House, Fulbrook Road, Cambridge. (1920.)

275. Robinson, Wilfred, Department of Botany, Prof. of University College of Wales, Aberystwyth. (1923.)
276. Robson, Mr R., M.Sc., F.Z.S., Writtle, Chelmsford, Essex. (1914.)
277. Rolfe, Mr F. W., Colonial and Indian Collections, Imperial Institute, London, S.W. 7. (1923.)
278. Rolfe, Mr R. T., F.I.C., 76, Spenser Road, Bedford. (1924.)
279. Roper, Miss Ida M., F.L.S., 4, Woodfield Road, Redland, Bristol. (1921.)
280. Rothamsted Experimental Station, Department of Mycology, Harpenden, Herts. (1923.)
281. Rushton, Mr W., A.R.C.S., D.I.C., St Thomas's Hospital Medical School, Albert Embankment, London, S.E. 1. (1914.)
282. Rutgers College and State University of New Jersey, Library, New Brunswick, New Jersey, U.S.A. (1922.)
283. Ryan, Mr G. M., F.L.S., 35, Ladbroke Gardens, London, W. 11. (1923.)
284. St Paul, Minnesota, U.S.A., The Library, Department of Agriculture, University Farm. (1920.)
285. Salisbury, Mr E. J., D.Sc., F.L.S., Botanical Department, University College, Gower Street, London, W.C. 1. (1921.)
286. Salmon, Professor E. S., F.L.S., South-Eastern Agricultural College, Wye, Kent. (1922.)
287. Sampson, Miss K., B.Sc., University College, Aberystwyth, North Wales. (1920.)
288. Samuel, Mr Geoffrey, The University of Adelaide, South Australia. (1923.)
289. Sanderson, Mr A. R., F.L.S., Research Laboratory, Rubber Growers' Association, Petaling, Federated Malay States. (1921.)
290. Schinz, Professor Dr, Botanical Garden and Museum, Zurich, Switzerland. (1921.)
291. Science Library, South Kensington, London, S.W. 7. (1924.)
292. Scott, Mr W. W., 23, Wood Lane, Highgate, London, N. 6. (1922.)
293. Searle, Mr G. Odell, B.Sc. (Agric.), Research Botanist, Linen Industry Research Association, Glenmore House, Lambeg, Lisburn, Ireland. (1920.)
294. Sharpe, Mr C. J., Brambleside, Manor Road, Sidcup. (1905.)
295. Sharples, Mr A., A.R.C.S., D.I.C., Mycologist, Department of Agriculture, Kuala Lumpur, Federated Malay States. (1924.)
296. Shaw, Mr F. J. F., D.Sc., A.R.C.S., F.L.S., Imperial Agricultural Research Institute, Pusa, Bihar, India. (1920.)

297. Small, Mr W., M.B.E., M.A., Ph.D., B.Sc., Mycologist, Royal Botanic Gardens, Peradeniya, Ceylon. (1915.)
298. Smith, Mr Alexander, The Botany School, Cambridge. (1924.)
299. Smith, Mr F. E. V., B.Sc., Ministry of Agriculture, Pathological Laboratory, Milton Road, Harpenden, Herts. (1922.)
300. Smith, Miss K. E., 64, Coton Road, Nuneaton. (1913.)
301. Smith, Mr Noel J. G., 9, Braidburn Crescent, Edinburgh. (1924.)
302. Smith, Miss S. Somerford, 6, Barbourne Terrace, Worcester. (1923.)
303. Solberg, Miss Louise, Kobbervik Drammen, Norway. (1923.)
304. South London Botanical Institute, Tulse Hill, Herne Hill, London, S.E. 24. (1921.)
305. Southwell, Mr Herbert, A.R.C.S., 158, Lincoln Road, Peterborough. (1923.)
306. Stakman, Professor E. C., University of Minnesota, Minneapolis, Minn., U.S.A. (1922.)
307. Stansfield, Miss O. P., M.Sc., Milton Mount College, Crawley, Sussex. (1922.)
308. Stationery Office, H.M., Superintendent of Publications, Book Dept., Westminster, S.W. 1. (2 subscriptions.) (1920.)
309. Stelfox, Mrs, "Mayfield," Clareville Road, Rathgar, Dublin. (1925.)
310. Stirrup, Mr H. H., M.Sc., Midland Agricultural College, Sutton Bonington, Loughborough. (1922.)
311. Storey, Mr H. H., B.A., Natal Herbarium, Durban, South Africa. (1922.)
312. Sutherland, Mr G. K., M.A., D.Sc., F.L.S., 110, Brackenbury Road, Moor Park, Preston. (1914.)
313. Swansea Field Naturalists' Society, c/o Mr Alan Stuart, M.Sc., University College, Singleton, Swansea, South Wales. (1924.)
314. Swanton, Mr E. W., A.L.S., Educational Museum, Haslemere, Surrey. (1899.)
315. Swedish Academy of Sciences, Royal, Stockholm, Sweden. (1919.)
316. Sydney, Australia, The Librarian, University of. (1922.)
317. Tabor, Mr R. J., B.Sc., F.L.S., Botanical Department, Imperial College of Science, South Kensington, London, S.W. 7. (1914.)
318. Tagg, Mr H. F., F.L.S., Royal Botanic Garden, Edinburgh. (1921.)
319. Tandy, Mr Geoffrey, Department of Botany, British Museum, Cromwell Road, South Kensington, London, S.W. 7. (1925.)
320. Taylor, Miss B. K., 98, Cheyne Walk, Chelsea, London, S.W. 3. (1910.)

321. Taylor, Mr G. Crosbie, "The Garden," 20, Tavistock Street, Covent Garden, London, W.C. 2. (1924.)
322. Tennessee, University of, Agricultural Experiment Station, Library, Knoxville, Tennessee, U.S.A. (1926.)
323. Thomson, Miss N. L., 4, Burton Court, London, S.W. 3. (1926.)
324. Tomkins, Mr R. G., B.A., Trinity College, Cambridge. (1925.)
325. Toronto, University of, Librarian, Toronto, Canada. (1919.)
326. United States Department of Agriculture, c/o W. Wesley & Son, 28, Essex Street, Strand, London, W.C. 2. (1907.)
327. Vines, Professor S. H., M.A., D.Sc., F.R.S., F.L.S., Langstone, Exmouth, Devon. (1915.)
328. Wadham, Professor S. M., M.A., Department of Agriculture, The University, Melbourne, Victoria, Australia. (1922.)
329. Wager, Mr H., D.Sc., F.R.S., F.L.S., 4, Bank View, Chapel Allerton, Leeds. (1896.)
330. Wakefield, Miss E. M., M.A., F.L.S., Herbarium, Royal Botanic Gardens, Kew. (1911.)
331. Waldie, Mr J. S. L., B.Sc., C.D.A., Royal Botanic Garden, Edinburgh. (1925.)
332. Wallis, Mr A., Westacre, Station Road, Kettering. (1921.)
333. Ward, Mr F. S., Department of Agriculture, Kuala Lumpur, Federated Malay States. (1925.)
334. Ware, Mr W. M., M.Sc., South-Eastern Agricultural College, Wye, Kent. (1924.)
335. Washington, Library, State College of Washington, Pullman, Wash., U.S.A. (1924.)
336. Watson, Mr W., D.Sc., A.L.S., Taunton School, Taunton. (1923.)
337. Welsford, Miss E. J., Mycologist, Department of Agriculture, Zanzibar, East Africa. (1924.)
338. Westerdijk, Professor Johanna, Javalaan 4, Baarn, Holland. (1923.)
339. Weston, Mr W. A. R. Dillon, B.A., School of Agriculture, Cambridge. (1923.)
340. Whetzel, Professor H. H., M.A., New York State College of Agriculture, Cornell University, Ithaca, N.Y., U.S.A. (1914.)
341. Whitaker, Mr F. Owen, 89, Eccleston Square, London, S.W. 1. (1921.)
342. Whitehead, Mr T., A.R.C.S., University College of North Wales, Bangor. (1920.)
343. Williams, Professor J. Lloyd, D.Sc., F.L.S., Botanical Department, University College of Wales, Aberystwyth. (1921.)
344. Williamson, Mrs H. S., B.Sc., 3, Verulam Buildings, Gray's Inn, London, W.C. 1. (1921.)
345. Wilson, Mr Malcolm, D.Sc., A.R.C.S., F.L.S., Royal Botanic Garden, Edinburgh. (1921.)

- 346. Wiltshire, Mr S. P., M.A., Imperial Bureau of Mycology, 17, Kew Green, Kew, Surrey. (1920.)
- 347. Wiltshire, Mrs S. P., 24, Lawn Crescent, Kew Gardens, Surrey. (1925.)
- 348. Winsor, Mr A. P., A.R.C.Sc.I., Ministry of Agriculture and Fisheries, 10, Whitehall Place, London, S.W. 1. (1923.)
- 349. Wisconsin, The Library, University of, Madison, Wis., U.S.A. (1923.)
- 350. Wolf, Mr B. L., N.D.A., 55, Catharine Street, Salisbury. (1923.)
- 351. Wood, Mr N. J., The Cottage, Barcombe, near Lewes, Sussex. (1923.)
- 352. Woodcock, Mr A. J. A., M.Sc., F.E.S., Clifton Manor, York. (1926.)
- 353. Woodward, Mr R. C., B.Sc., School of Rural Economy, Oxford. (1924.)
- 354. Woolhope, The Naturalists' Field Club, Hereford, c/o Mr C. S. Scobie, 2, Offa Street, Hereford. (1896.)
- 355. Worcestershire Naturalists' Field Club, c/o Mr W. J. Else, Victoria Institute, Worcester. (1921.)
- 356. Wormald, Mr H., D.Sc., A.R.C.S., Research Station, East Malling, Kent. (1921.)
- 357. Wright, Mr E. Barton, B.Sc., Oakleigh, Godstone, Surrey. (1926.)

## RULES.

### *Society's name and objects.*

1. The Society shall be called "The British Mycological Society," and its objects shall be the study of Mycology in all its branches.

### *Members of Society.*

2. The Society shall consist of Honorary Members, Foundation Members and Ordinary Members; the number of Honorary Members shall be limited to 20, and that of Foundation Members to 100\*, but the number of Ordinary Members shall be unlimited.

### *Honorary Members.*

3. Honorary Members shall be persons of pre-eminence in Mycology, or who have rendered special service to the Society.

### *Foundation Members.*

4. Foundation Members shall be those Members or Societies who joined the Society previous to the limit of 100 Members having been attained\*.

### *Officers.*

5. The Officers of the society shall consist of a President, one or more Vice-Presidents, Treasurer, Secretaries, and Editor or Editors. They shall be elected annually at the Annual General Meeting of the Society.

### *Government of Society.*

6. The government of the Society shall be vested in a Council, which shall consist of the President and other Officers for the time being, together with two or more other Members who shall be elected annually at the General Meeting, and one-half of whom shall retire each year and not be eligible for immediate re-election. The Members to retire shall be those who have been longest in office or, in case of equality, shall be determined by ballot. Ex-Presidents are *ex officio* Members of the Council.

Every Meeting of the Council shall be duly summoned by the Hon. Secretary by at least seven days' notice in writing to each Member of the Council.

\* The limit of 100 Foundation Members was reached 22nd Oct. 1903.



*Period of Office.*

7. The Officers and Council shall hold office as from the 1st of January following their election.

*Election of Members.*

8. Honorary Members shall only be elected at a Meeting of the Society by a majority of the Members then present.

All Ordinary Members shall be proposed and seconded respectively by existing Members, who shall sign a certificate (see appendix) of recommendation, one at least of the proposers so certifying from personal knowledge. Every candidate for election shall sign an undertaking to abide by the Rules if elected (see appendix). They shall be elected by a majority of the Members present at any meeting of the Society or by the President and Officers of the Society.

*Subscription.*

9. All Ordinary Members and Societies shall pay an annual subscription of one pound, and Foundation Members five shillings, due on the 1st of January in each year. Honorary Members shall be exempt from any annual subscription.

Any Member wishing to retire from the Society shall give notice to the Hon. Secretary or Treasurer in writing before the 1st of December of the previous year.

*Meetings.*

10. The Society shall hold one or more Meetings annually, at a place and time determined by the Members at the preceding Annual General Meeting, or by the Council. The Annual General Meeting for the election of Officers and the transaction of other business shall coincide with the Autumn Foray.

*Accounts.*

11. At the Annual General Meeting of the Society in each year the Hon. Treasurer shall present duly audited accounts.

*Alteration of Rules.*

12. The Rules shall not be altered except by a two-thirds majority of the Members present at an Annual General Meeting. A printed copy shall be sent to every Member of the Society on election, and in the event of alteration to all Members.

## APPENDIX.

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### *Form of proposal for Ordinary Membership of the British Mycological Society.*

---

of .....

.....

being desirous of becoming an Ordinary Member of the British Mycological Society, we, the undersigned Members of the Society, certify that we consider h            to be a desirable Member of the Society, and beg to recommend h            for election.

Dated this                      day of                      19

.....(From personal knowledge).

---

### *Certificate to be signed by the Candidate.*

I hereby certify that I desire to become an Ordinary Member of the British Mycological Society and that I will abide by the Rules if elected.

.....



# INDEX

New species are indicated by an asterisk

- \**Acremonium griseum* Petch, 262
- "Additions and corrections to entomogenous fungi, II," by T. Petch, 258, text-fig.
- Aegerita*, 59
- A. Webberi* Fawcett, 52, Pl. I
- Fungi parasitic on, 65
- Agropyron repens*, 139
- Agrostis vulgaris*, 139
- Alcock, N. L. "Successional disease in plants as shown in willow rods," 161, text-fig.
- Alisma Plantago-aquatica*, 198
- Animals, Toxicity of spores of *Tilletia Tritici* to, 82
- Anona muricata*, 119
- Antirrhinum majus*, Life-history of fungus parasitic on, 169
- Apple, Dying-back of branches of, associated with a pycnidial fungus, 213
- Apples, Core-rot and premature fall of, associated with *Sclerotinia fructigena*, 155
- Arcyria pomiformis* (Leers) Rost., 22
- Arrhenatherum avenaceum*, 139
- Artocreas Micheneri* B. & C., 67
- A. poroniaeforme* B. and Br., 67, Pl. II, fig. 1, Pl. III, figs. 1-5 and text-fig.
- Ascomycetes in *Penicillium*, 108
- Ash, *Fomes annosus* on, 13
- Aspicilia calcarea* Sommerf., 19
- Avocado pear, *Colletotrichum* on, 119
- Bacidia luteola* Mudd, 20
- Badhamia panicea* (Fr.) Rost., 23
- Bananas, *Colletotrichum* on, 120
- Barley, 139
- Bartlett, A. W., "On a new species of *Urophycitis* producing galls on *Lotus corniculatus* Linn.," 266, Pls. XI-XIV
- Beans, *Colletotrichum* on, 117
- Bellis perennis*, 198
- Biatorella flava* A. L. Sm., 20
- B. privigna* A. L. Sm., 20
- Biatorina Bouteillei* Arn., 20
- B. graniformis* A. L. Sm., 20
- "Black Rust in Scotland," by I. Maxwell and G. B. Wallace, 138, Pl. IV
- Boletus elegans* (Schum.) Fr., 12
- B. viscidus* (Linn.) Fr., 12
- Botryodiplodia Theobromae* Pat., 281
- "*Botryodiplodia* sp. on *Choisya ternata* in England," by R. C. Woodward, 281, text-fig.
- Botrytis necans* Massee, 263
- B. Rileyi* Farlow, 264
- Bromus sterilis*, 139
- Brooks, F. T., Southee, E. A., and "Notes on a pycnidial fungus associated with a dying-back of apple branches," 213, 7 text-figs.
- Buckley, W. D., 5
- Buddin, W., and Wakefield, E. M., "On the life-history of a fungus parasitic on *Antirrhinum majus*, with some remarks on the genus *Heterosphaeria*," 169; Supplementary note, 186, 8 text-figs.
- Cacao, *Colletotrichum* on, 116
- Callitriche stagnalis*, 198
- Carica Papaya, 119
- \**Cephalosporium falcatum* Petch, 259
- Cercospora Opuli* (Fuck.) v. H., 13
- Cercospora* *Antirrhini* Wakef., 171
- Cetraria glauca* Ach., 19
- Cheesman bequest, 4
- Cheesman, W. N., obituary, 1
- Chillies, *Colletotrichum* on, 118
- Choisya ternata*, *Botryodiplodia* on, 281
- Ciliaria scutellata* (Linn.) Quél., 5
- C. trechispora* (B. & Br.) Boud., 5
- Citrus, *Colletotrichum* on, 118
- Clathroporina calcarea* Wats., 10
- Coffea arabica*, 115
- Coffee berries, Preliminary account of disease of green, 145
- Coffee, *Colletotrichum* on, 115
- Colletotrichum coffeanum* Noack, 146
- Colletotrichum*, On the occurrences of a species of, 112
- Cook, W. R. Ivey, "The genus *Ligniera* M. & T.," 196, Pls. VIII and IX
- Conidial forms of *Septobasidium*, 62, text-fig.
- Coprinus sterquilinus*, Inheritance of spore size in, 219
- \**Coremium breve* Petch, 259
- \**C. gracile* Petch, 260
- \**C. pulcherrimum* Petch, 259

- Core-rot of apples associated with  
*Sclerotinia fructigena*, 155  
*Corticium subgiganteum* Berk., 69  
 Cotton, *Colletotrichum* on, 116  
*Cribaria piriformis* Schrad., 22  
*Cryptomyces maximus* (Fr.) Rehm,  
 165  
*Cryptosporiopsis* Petch, 167  
*Cryptothecia* Stirton apud A. Zahlb.,  
 192  
 \**C. conferta* A. L. Sm., 194, Pl. VII,  
 fig. 6  
 \**C. Stirtonii* A. L. Sm., 193, Pl. VII,  
 fig. 4  
*C. subnidulans* Stirt., 189  
*C. subnidulans* (Stirt.) A. L. Sm., 192,  
 Pl. VII, fig. 1  
 \**C. subtecta* (Stirt.) A. L. Sm., 194,  
 Pl. VII, fig. 5  
 "Cryptotheciaceae, a family of primi-  
 tive Lichens," by A. Lorrain  
 Smith, 188, Pl. VII  
 \*Cryptotheciaceae A. L. Sm., 190  
*Cytospora fructorum* E. & E.  
 March., 218, text-figs.
- Dactylis glomerata*, 139  
*Daldinia concentrica* (Bolt.) Ces. &  
 de Not., 5  
*Dasyscypha caulicola* (Fr.) Boud., 5  
*Dermatea Cerasi* (Pers.) Boud., 5  
*Dermatocarpon miniatum* Th. Fr., 19  
 Derx, H. G., "Heterothallism in the  
 genus *Penicillium*," 108  
*Diderma lucidum* B. & Br., 24  
*D. ochraceum* Hoffm., 24  
*Didymium Clavus* (A. & S.) Rost.,  
 22  
*D. squamulosum* (A. & S.) Fr., 22  
*Disciotis venosa* (Pers.) Boud., 5  
 Disease of green coffee berries in  
 Kenya Colony, Preliminary ac-  
 count of, 145  
 Disease, Successional, in plants, as  
 shown in willow rods, 161  
 Dobson, Norman, "The toxicity of  
 the spores of *Tilletia Tritici* to  
 animals," 82  
 Dowson, W. J., "On a core-rot and  
 premature fall of apples as-  
 sociated with *Sclerotinia fructi-*  
*gena*," 155, Pl. V and text-fig.  
 Dublin Foray, 11  
 — Lichens of, 18  
 — Mycetozoa of, 22  
 Dyes, Lichen, 45  
 Dying-back of apple branches, Notes  
 on pycnidial fungus associated  
 with, 213  
 "Ecology of Fungi," by J. Rams-  
 bottom, 12
- Empusa Lecanii* Zimm., Studies in  
 entomogenous fungi, XI, 254, text-  
 fig.  
 "Entomogenous fungi, Additions and  
 corrections, II," by T. Petch, 258,  
 text-fig.  
 Entomogenous fungi, Studies in, IX,  
*Aegerita*, 50; X, *Verticillium* sp.,  
 251; XI, *Empusa Lecanii* Zimm.,  
 254  
*Festuca elatior*, 139  
 Figs, *Colletotrichum* on, 120  
*Fomes annosus* Fr., 13  
 Foray, Dublin, 11; Mycetozoa of, 22  
 — Tintern, p. 5  
 Fungi, Ecology of, 12  
 — Entomogenous, Studies in, IX,  
*Aegerita*, 50; *Verticillium*, 251;  
 XI, *Empusa Lecanii* Zimm., 254  
 — parasitic on *Aegerita Webberi*,  
 65  
 Fungi, Taxonomy of, 25  
 "Fungus associated with dying-back  
 of apple branches," by E. A.  
 Southey and F. T. Brooks, 213,  
 7 text-figs.  
 \**Fusarium entomophilum* Petch, 260  
*Fusicladium saliciperdu* (All. &  
 Tub.) Tub., 163, text-fig.
- Galls on *Lotus corniculatus*, 266  
*Ganoderma applanatum* (Pers.) Pat.,  
 13  
 Green coffee berries in Kenya Colony,  
 Preliminary account of disease  
 of, 145  
 Ground-nut leaves, *Colletotrichum* on,  
 120
- Hanna, W. F., "The inheritance of  
 spore size in *Coprinus sterquilii-*  
*nus*," 219  
*Hebeloma fastibile* Fr., 12  
*Hemitrichia clavata* (Pers.) Rost., 23  
*Heteropatella* on *Antirrhinum*, 169  
 \**Heteropatella Antirrhini* Budd. &  
 Wakef., 188, text-figs.  
 H. Bonordeni (Hazsl.) Lind., 184  
 H. *Hendersonioides* Fautr. & Lamb.,  
 184  
 H. *lacera* Fuck., 182  
*Heterosphaeria Linariae* (Rabh.)  
 Rehm, 179  
 H. *Patella* Grev., 6, 179  
 "Heterothallism in the genus *Peni-*  
*cillium*," by H. G. Derx, 108  
*Hevea brasiliensis*, *Colletotrichum* on,  
 118  
 \**Hirsutella nodulosa* Petch., 261,  
 text-fig.  
*Humulus Lupulus*, 106

- "Inheritance of spore size in *Coprinus sterquilinus*," by W. F. Hanna, 219
- Iris Pseud-acorus, 198
- Irish mycetoza, Notes on, 23
- Isaria stellata Cke., 264
- Jasione montana, 6
- Juncus articulatus, 198
- Knight, H. H., "Lichens of the Tintern Foray," 9
- Knowles, M. C., and Smith, A. L., "Lichens of the Dublin Foray," 18
- Lachnobolus congestus (Somm.) List., 23
- Lamproderma columbinum Rost., v. brevipes, G. List., 24
- Lecanora rugosa Nyl., 20
- L. varia Ach., 19
- Lecidea parasema Ach., 19
- Lepiota castanea Quél. Pl. vi
- L. echinella Quél. & Bern., Pl. vi
- L. gracilentia (Krombh.) Fr., Pl. xv
- L. gracilis (Quél.) Rea, Pl. vi
- L. lenticularis (Lasch) Cke., 11
- L. pratensis (Fr.) Rea, Pl. vi
- Leptonia chalybea (Pers.) Fr., 11
- Leptospora caudata Fuck., 6
- "Lichen Dyes," by A. Lorrain Smith, 45
- "Lichens of the Dublin Foray," by A. L. Smith and M. C. Knowles, 18
- "Lichens of the Tintern Foray," by H. H. Knight, 9
- Ligniera Alismatis Schw., 197
- L. Bellidis Schw., 211
- L. graminis Schw., 211
- L. Junci (Schw.) Maire & Tison, 211, Pls. viii and ix
- L. Menthae Schw., 211
- L. pilorum, Fron & Gaill., 211
- L. radicalis Maire & Tison, 211
- L. verrucosa Maire & Tison, 211
- Lister, G., "Mycetoza of the Dublin Foray," 20
- "Notes on Irish Mycetoza," 23
- Lobaria pulmonaria Hoffm., 19
- Lolium perenne, 139
- Lotus corniculatus Linn., A new species of Urophlyctis producing galls on, 266
- Lycogala epidendrum (Linn.) Fr., 22
- L. fragilis Hoffm., 263
- Mango, Colletotrichum on, 118
- Mason, E. W., "On two species of Tolyposporium Woronin recorded on cultivated Sorghum," 284
- "Matula," by T. Petch, 67, Pls. II and III
- Matula Rompelli Rick., 77
- Maxwell, I., and Wallace, G. B., "Black Rust in Scotland," 138, Pl. IV
- McDonald, J., "Preliminary account of a disease of green coffee berries in Kenya Colony," 145
- Melanosphaeria circumdata Saw., 263
- Members, List of, 287
- Mentha Pulegium, 178
- Michenera artocreas B. & C., 67, text-fig.
- Micropodia pteridina (Karst.) Boud., 5
- Microsphaeria Euxynymi (DC.) Sacc., 13
- Milesina Kreigeriana Magn., 5
- M. Polystichi (Wincl.) Grove, 5
- M. Scolopendrii (Fuck.) Jaap., 5, 13
- Mitrophora hybrida (Sow.) Boud., 5
- \*Moniliopsis rigida Petch, 258
- Mycelial invasion of the host plant by Pseudoperonospora Humuli, 91
- Mycetoza, Notes on Irish, 23
- of the Dublin Foray, 22
- "Mycology and Human Affairs," by J. Ramsbottom, 12
- Mycosphaerella hedericola (Desm.) Lindau, 6
- Myxosporium scutellatum (Atth.) Petrak, 166
- Nectria mammoidea Phill. & Plowr., 6
- N. sanguinea (Sibth.) Fr., 5
- Oats, 139
- Obituary, W. N. Cheesman, 1
- Oil-palm, Colletotrichum on, 120
- Oospora Aphidis Cke. & Masee, 265
- Opographa paraxanthodes Nyl., 9
- Papaw, Colletotrichum on, 119
- Parmelia saxatilis Ach., 19
- Penicillium, Heterothallism in, 108
- Penicillium luteum Rich., 108
- P. luteum (Zukal?) Wehm., 109
- P. Wortmanii Klöck., 109
- Peniophora Haegallae (B. & Br.) Cke., 78, Pl. II, fig. 2, Pl. III, fig. 6
- Perichaena corticalis (Batsch) Rost., 23
- Peronospora Humuli, Mycelial invasion of the host plant by, 91, 2 text-figs.
- Persea gratissima, 119
- Pertusaria globulifera Nyl., 19
- P. pertusa Dalla Torre & Sarnth., 19
- Petch, T., "Matula," 67, Pls. II and III
- "Rhacophyllus B. & Br.," 238, Pl. x and 2 text-figs.
- "Studies in entomogenous fungi, additions and corrections, II,"

- 258; "IX, *Verticillium* sp.," 251;  
 "IX, *Aegerita*," 50; "XI, *Empusa*  
*Lecanii* Zimm.," 254  
*Pethybridge*, G. H., elected President,  
 12  
*Phacidium* *Patella* (Tode) Fr.  $\alpha$   
 alpestre Fr., 183;  $\beta$  campestre  
 Fr., 183  
*Phaseolus* *vulgaris*, 117  
*Phleum* *pratense*, 139  
*Phyllosticta* *hedericola* Dur. & Mont.,  
 6  
*Phyalospora* *gregaria* Sacc., 164  
*Physarum* *brunneolum* (Phill.) Mass.,  
 24  
*P. pulcherripes* Peck., 24  
*Physcia* *orbicularis* Dalla Torre &  
 Sarnth., 19; var. *virella* Dalla  
 Torre & Sarnth., 19  
*P. tribacia* Nyl., 19  
*Picea* *pungens*, 12  
*Placynthium* *nigrum* S. F. Gray, 19  
*Plantago* *major*, 197  
 Plants, Successional disease in, 161  
*Pleurotus* *geogenius* (DC.) Fr., 11  
*Poa* *annua*, 197  
*P. nemoralis*, 139  
*Polyporus* *hispidus* (Bull.) Fr., 13  
*P. squamosus* (Huds.) Fr., 13  
*Potamogeton* *natans*, 198  
 Premature fall of apples associated  
 with *Sclerotinia* *fructigena*, 155  
 Presidential Address by J. Rams-  
 bottom, "The taxonomy of  
 fungi," 25  
 Proceedings, 168  
*Propolis* *faginea* (Schr.) Karst., 5  
*Prunus* *Avium*, 5  
*Psaliota* *haemorrhoidaria* Kalchbr.,  
 11  
 "Pseudoperonospora *Humuli* and its  
 mycelial invasion of the host  
 plant," by W. M. Ware, 91,  
 2 text-figs.  
*Puccinia* *Buxi* DC., 13  
*P. graminis-Agrostidis* Eriks., 138  
*P. graminis-Avenae* Eriks. & Henn.,  
 138  
*P. graminis-compacti* Stak. & Piem.,  
 138  
*P. graminis* *Phlei-pratensis* Stak. &  
 Piem., 138  
*P. graminis* *Poa* Eriks. & Henn., 138  
*P. graminis* *Secalis* Eriks. & Henn., 138  
*P. graminis* *Tritici* Eriks. & Henn.,  
 138  
*P. tumida* Grev., 5  
*Pyrenopeziza* *Jasionis* Romell, 6  
*Pyrenula* *nitida* Ach., 19  
 Ramsbottom, J., "Ecology of fungi,"  
 12  
 Ramsbottom, J., "Mycology and  
 Human affairs," 12  
 — Presidential address, "The tax-  
 onomy of fungi," 25  
 — W. N. Cheesman, Obituary, 1  
*Ranunculus* *aquatilis*, 198  
*R. circinnatus*, 197  
 Raspberries, *Colletotrichum* on, 120  
 Rea, E. A. and V., Plates VI and XV  
 Reviews:  
 J. Read, "A Text Book of Organic  
 Chemistry, Historical, Struc-  
 tural and Economic," 168  
 R. T. and F. W. Rolfe, "The Ro-  
 mance of the Fungus World,"  
 168  
 "Rhacophyllus B. & Br." by T.  
 Petch, 238, Pl. x and 2 text-figs.  
*Rhacophyllus* *lilacinus* B. & Br., 238  
*Rhinotrichum* *album* Petch, 258  
*Rhizocarpon* *petraeum* Massal., 10  
*Rhizosphaera* *Kalkhoffii* Bub., 12  
 Rust, Black, in Scotland, 138  
 Rye, 139  
*Salix* spp., 163  
*Scleroterris* *fuliginosa* (Pers.) Karst.,  
 165  
*Sclerotinia* *fructigena* associated with  
 core-rot and premature fall of  
 apples, 155  
*Septobasidium*, Conidial forms of, 62,  
 text-fig.  
*Septobasidium* *albidum* Pat., 64  
*S. lanosum* Pat., 63  
*S. Thwaitesii* (B. and Br.) Pat., 65  
*Septoria* *Hederae* Desm., 6  
*Sirospora* *chlorostoma* Petch, 65  
 Small, W., "On the occurrences of a  
 species of *Colletotrichum*," 112  
 Smith, A. Lorrain, "Cryptotheci-  
 aceae, a family of primitive  
 lichens," 188, Pl. VII  
 — "Lichen dyes," 45  
 Smith, A. L., and Knowles, M. C.,  
 "Lichens of Dublin Foray," 18  
*Sorghum*, Two species of *Tolypos-  
 porium* Woronin recorded on,  
 284  
*Sorosphaera* *graminis* Schw., 211  
*S. Junci* Schw., 211  
*Sorosporium* *Ehrenbergii* Kühn, 284  
*Soursop*, *Colletotrichum* on, 110  
 Southee, E. A. and Brooks, F. T.,  
 "Notes on a pycnidial fungus  
 associated with a dying-back of  
 apple branches," 213, 7 text-figs.  
*Sphaeria* *Patella* Pers., 182  
 Spore size, inheritance of, in *Coprinus*  
*sterquilinus*, 219  
 Spores of *Tilletia* *Tritici*, Toxicity of,  
 to animals, 82

- \**Sporotrichum album* Petch, 262  
 \**Stilbella coccorum* Petch, 260  
 \**Stilbum* (*Stilbella*) *coccorum* Petch, 260  
 \**Stirtonia* A. L. Sm., 195  
 \**S. dubia* A. L. Sm., 195, Pl. VII, fig. 8  
 \**S. obvallata* A. L. Sm., 195, Pl. VII, fig. 7  
 "Studies in entomogenous fungi, IX, *Aegerita*," by T. Petch, 50; "X, *Verticillium* sp.," 251; "XI, *Empusa Lecanii* Zimm.," 254  
 "Successional disease in plants as shown in willow rods," by N. L. Alcock, 161, text-fig.  
*Synchytrium Mercurialis* (Lib.) Fuck., 5  
*S. Taraxaci* de By. & Woron., 5  
  
*Taphridium umbelliferarum* (Rostr.) Juel, 6  
*Taxonomy of Fungi*, 25  
*Tea*, *Colletotrichum* on, 118  
*Ticothecium rimosicolum* Arn., 10  
*Tilletia Tritici*, Toxicity to animals of spores of, 82  
*Tintern Foray*, 5  
 — Lichens of, 9  
*Tolyposporium Woronin*, two species recorded on cultivated *Sorghum*, 284  
*T. Ehrenbergii* (Kühn) Pat., 284  
*T. filiferum* Busse., 284  
*T. Volkensii* Henn., 285  
 Toxicity of spores of *Tilletia Tritici* to animals, 82  
*Tremellodon gelatinosum* (Scop.) Pers., 12  
*Trichia contorta* (Ditm.) Rost. v. *inconspicua* List., 23  
*T. decipiens* (Pers.) Macbr., 23  
 \**Tubercularia epimyces* Petch, 66, 263  
  
*Urophlyctis*, new species producing galls on *Lotus corniculatus* Linn., by A. W. Bartlett, 266  
 \*U. *Potteri* Bartlett, 279, Pl. XI-XIV  
*U. spp.*, 278  
  
*Valeriana dioica*, 200  
*Vanilla*, *Colletotrichum* on, 119  
*Vermicularia trichella* Fr., 6  
*Veronica Beccabunga*, 196  
*Verrucaria parva* Deak., 20  
*Verticillium spp.*, 251  
*V. heterocladium* Penz., 253, text-fig.  
*Viburnum Opulus*, 13  
*Voacanga Thouarsii*, *Colletotrichum* on, 120  
*Volutella ciliata* (A. & S.) Fr., 6  
  
*Wakefield*, E. M., Dublin Foray, 11  
 — Tintern Foray, 5  
*Wakefield*, E. M., Buddin, W. and, "On the life-history of a fungus parasitic on *Antirrhinum majus*, with some remarks on the genus *Heterosphaeria*," 169; Supplementary note, 186, 8 text-figs.  
*Wallace*, G. B., Maxwell, I. and, "Black Rust in Scotland," 138, Pl. IV  
*Ware*, W. M., "Pseudoperonospora *Humuli* and its mycelial invasion of the host plant," 91, 2 text-figs.  
*Willow rods*, Successional disease in, 161  
*Willow scab*, 163  
*Wheat*, 139  
*Woodward*, R. C., "A note on *Botryodiplodia* sp. on *Choisya ternata* in England," 281, text-fig.